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1. Perlman, H. H.: J. Pediat. 33: 114, 1948.

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The Journal of Allergy

VOL. 22 MARCH, 1951 NO. 2

Original Articles

THE EFFECT OF ACTH ON THE IMMEDIATE SKIN REACTION AND PASSIVE TRANSFER TEST IN MAN*

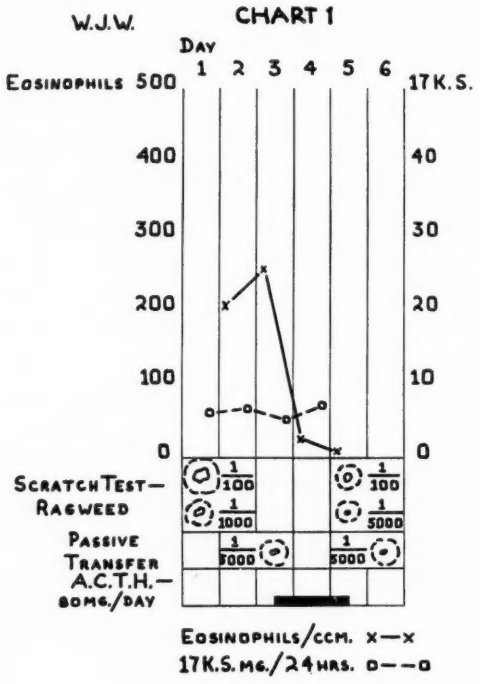
WILFRED LEITH, M.D., MARY J. GRAHAM, A.B., AND WALTER S. BURRAGE, M.D.
BOSTON, MASS.

IT HAS been demonstrated that ACTH (adrenocorticotropic hormone) is of value in the treatment of allergic disease. Symptoms of rhinitis, wheezing, and skin irritation, as well as signs of nasal congestion, pulmonary rhonchi, and skin eruptions have all been alleviated or entirely dissipated by this mode of therapy.[1-4] The site of action of the adrenal hormones in disease states remains a matter of conjecture. The recorded observations upon the effect of ACTH on the immediate type of skin test have been few and inconclusive.[4, 5] It has been observed, however, that ACTH administration fails to alter the flare obtained by the intradermal injection of histamine. On the other hand, a positive tuberculin or delayed bacterial antigen skin test may be abolished by ACTH. Three to 21 days of therapy are required before this effect is obtained.[6]

We have attempted to study the immediate type of skin reaction and the passive transfer test (Prausnitz-Küstner)[7] before and after ACTH administration in pollen-sensitive hay fever patients. The patients studied have been followed for several years in the clinic or in private practice. They were all hay fever subjects under injection therapy, with symptoms only during their season, showing well-outlined positive skin tests to their offending pollens. The study was carried out during the months of May and June. Four patients had active symptoms of rhinitis while under observation; 2 were mild and 2 were severe—one of the 2 severely affected patients had wheezing and dyspnea as well. All of these patients were first observed for several weeks, during which period their clinical status was evaluated and scratch tests on the skin of the forearm were done. Only those positive skin reactions which were compatible with the clinical history were included. A negative Hinton test was obtained and a history of jaundice ruled out in all cases. A control period of 2 days was then instituted when daily urinary 17-ketosteroids were determined, and the circulating eosinophil counts were done. These were repeated during the following 2 days of ACTH therapy. The daily laboratory procedures were carried out to show whether or not an adrenal-stimulating effect was actually obtained during the ACTH administration.[8, 9]

*From the Allergy Clinic, Massachusetts General Hospital, Boston, Mass.

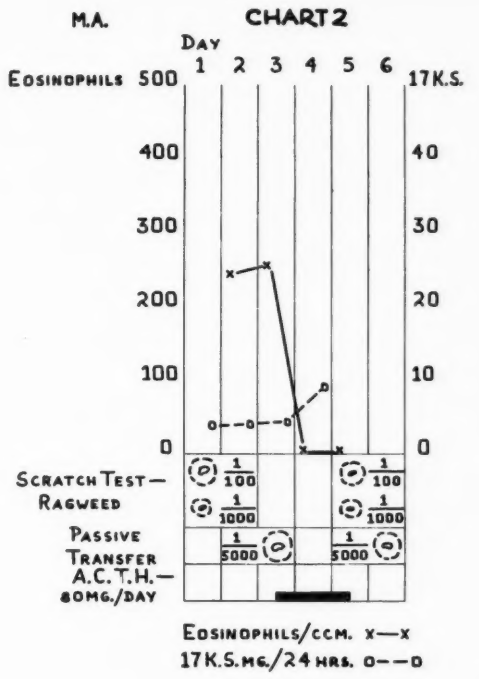
Ten milliliters of blood were withdrawn from the patients for a passive transfer test on the morning of the second day. Recipients were chosen who had negative personal and family histories of allergy, as well as negative intradermal tests to the incriminated antigen. On the afternoon of the second day, skin sites on the lateral aspect of the arms of the recipients were prepared by the intracutaneous injection of 0.1 ml. of the patient's serum. The passive transfer test was performed on the morning of the third day by injecting 0.05 ml. of the 1/5000 or 1/1000 dilution of the antigen into the previously prepared skin sites of the recipient. The reaction was read after 20 minutes.

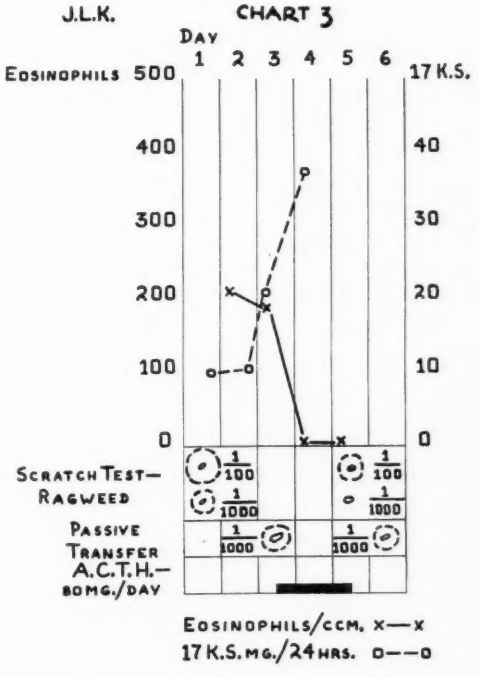


The ACTH* was started intramuscularly at noon of the third day in doses of 20 mg. and so continued every 6 hours for 8 doses, making a total of 160 mg. over a 2 day period. Blood for passive transfer and eosinophil count was taken and the skin tests were repeated on the morning of the fifth day, 4 hours after the last ACTH injection. The sites for passive transfer were prepared the afternoon of the fifth day in the same manner as described previously and were carried out upon the same recipient chosen for the control period. On the morning of the sixth day the passive transfer tests were done, repeating the procedure of the control period.

CASE 1.—W. L., a 31-year-old man, gave a typical history of uncomplicated ragweed hay fever. He showed clear-cut positive scratch tests to ragweed and to wormwood. No symptoms occurred during the period of observation. A passive transfer test to 1/5000 ragweed was positive. Eosinophil counts showed a marked fall while the 17-ketosteroids did not

*Armour standard preparation.

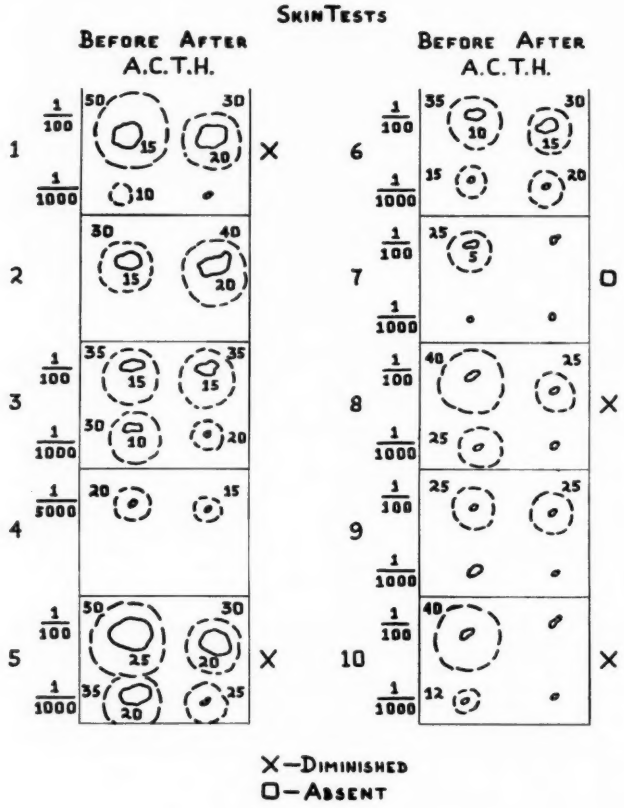




change with ACTH therapy. The scratch tests to ragweed and to wormwood diminished but were still present after ACTH therapy, while the passive transfer was present but appeared fainter.

CASE 2.—S. S., a 28-year-old man, presented a clinical picture of hay fever compatible with sensitiveness to trees, grasses, and ragweed. Good positive tests were demonstrated to timothy and ragweed. Severe rhinitis occurred during the period of study. Passive transfer to 1/5000 ragweed gave a wheal and surrounding erythema. ACTH administration caused a fall of eosinophils and a rise in 17-ketosteroids. Scratch and passive transfer tests were positive in both the control and treated periods although the scratch test was larger after treatment. Symptoms of rhinitis improved during ACTH therapy and continued so for 2 weeks, to be followed by a relapse of the hay fever.

CHART 4



CASE 3.—F. M. D., a 21-year-old man, had ragweed hay fever with definite positive scratch tests to ragweed. He was asymptomatic during the observation period. A passive transfer test to ragweed was positive. The eosinophils dropped while the 17-ketosteroids rose on ACTH administration. Neither the scratch test nor the passive transfer test was altered by ACTH therapy.

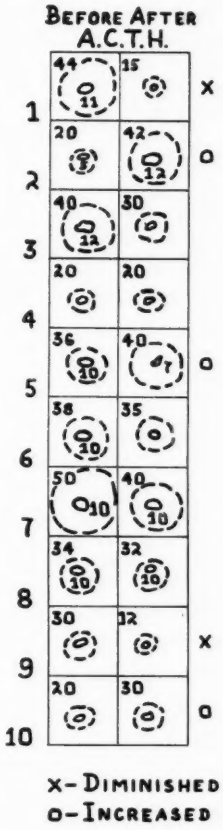
CASE 4.—L. H. B., a 37-year-old man, complained of hay fever and asthma continuously throughout the tree, grass, and ragweed seasons. Rhinitis and asthma were present during the period of observation. Scratch skin tests were inconclusive, but he showed positive intracutaneous tests to birch, to timothy, and to ragweed. A passive transfer test to ragweed was positive but faint. ACTH therapy was followed by a fall of eosinophils. The 17-ketosteroids failed to show an appreciable rise after ACTH. Although the rhinitis disappeared under treatment, the mild wheezing and dyspnea continued. The passive transfer test remained faintly positive as did the intracutaneous tests.

CASE 5.—W. J. W., a 35-year-old man, had grass and ragweed hay fever. Some rhinitis was present. Positive scratch tests were demonstrated to ragweed and to grasses. The passive tranfer test to ragweed was positive. The eosinophil count dropped after ACTH while the 17-ketosteroids failed to show a rise. Neither the passive transfer nor the scratch test altered appreciably. Symptoms of hay fever were alleviated.

CASE 6.—M. A., a 39-year-old female nurse with ragweed hay fever, showed a positive scratch test response. The passive transfer test was also positive; no symptoms were present. ACTH was followed by a fall of eosinophils while the 17-ketosteroids rose on the fourth day. The scratch and passive transfer tests did not change.

CHART 5

PASSIVE TRANSFER TEST



CASE 7.—F. E., a 44-year-old housewife with ragweed hay fever, showed positive scratch tests and a faintly positive passive transfer to ragweed. She was asymptomatic. Circulating eosinophils dropped with ACTH and the 17-ketosteroids rose on the fourth day. The scratch tests were negative following hormone injection, although the passive transfer test remained faint.

CASE 8.—J. L. K., a 37-year-old man with clinical ragweed and birch hay fever, showed positive scratch tests but only a faint passive transfer reaction. No symptoms were noted while under observation. ACTH therapy was followed by a drop in eosinophils and a rise in 17-ketosteroids. The scratch tests were somewhat diminished while the passive transfer test remained faint.

CASE 9.—D. B., a 33-year-old man with tree, grass, and ragweed hay fever, complained of active symptoms of rhinitis. Scratch tests were only mildly positive, as were the passive transfer tests. Eosinophils dropped after ACTH while the 17-ketosteroids rose. Although the scratch tests did not alter with therapy, the passive transfer was markedly diminished. Symptoms of rhinitis improved markedly with ACTH.

CASE 10.—C. W., a 36-year-old man with a ragweed hay fever history, was symptom free. Scratch and passive transfer tests were definitely positive. Eosinophils fell during ACTH therapy; the 17-ketosteroids did not alter. The pollen dilution scratch tests diminished greatly while the passive transfer test remained the same.

DISCUSSION

The present series consists of 10 hay fever subjects, ranging from 21 to 44 years of age and made up of 8 men and 2 women. All these patients gave histories of pollen hay fever with well-defined symptoms and with positive pollen skin tests. ACTH therapy in doses of 80 mg. per day over a 2 day period was followed by a temporary disappearance of hay fever symptoms within a matter of hours in the 4 patients who had had active discomfort. This therapy failed to control the wheezing, however, in the one patient who had asthma

TABLE I

PATIENT	SEX	AGE	CIRCULATING EOSINOPHILS BEFORE ACTH		CIRCULATING EOSINOPHILS AFTER ACTH	
			48 HR.	24 HR.	24 HR.	48 HR.
W. L.	♂	31	133	68	1	11
S. S.	♂	28	430	428	91	10
F.M. D.	♂	21	68	59	3	2
L. H. B.	♂	37	471	734	168	55
W. J. W.	♂	35	204	256	26	9
M. A.	♀	39	244	255	0	0
F. E.	♀	44	41	33	8	3
J. K. K.	♂	37	209	190	1	0
D. B.	♂	33	166	240	5	1
C. W.	♂	36	362	256	175	48

during the study. All 10 subjects exhibited a well-defined drop in circulating eosinophils after ACTH administration, indicating an adrenal response (Table I). A rise in urinary 17-ketosteroids was shown in only 6 patients (Table II). Reactions to the skin tests were diminished in 4, abolished in one, increased in one, and unaltered in 4 subjects after ACTH treatment (Chart 4). It has been

TABLE II

PATIENT	SEX	AGE	URINARY 17-KETOSTEROIDS BEFORE ACTH		URINARY 17-KETOSTEROIDS AFTER ACTH	
			48 HR.	24 HR.	24 HR.	48 HR.
W. L.	♂	31	10.6	11.8	12.8	11.2
S. S.	♂	28	7	7.8	8.6	11.4
F. M. D.	♂	21	11.4	12	16	18
L. H. B.	♂	37	3	7.8	7.2	8.8
W. J. W.	♂	35	6.2	6.8	5.2	7
M. A.	♀	39	4.2	4.3	4.4	9.2
F. E.	♀	44	3.2	3.4	3.2	7.6
J. K. K.	♂	37	9.9	10.5	20.4	37.6
D. B.	♂	33	5.1	6	8.2	12
C. W.	♂	36	12.4	11.6	12.6	12.2

demonstrated, however, that the size of skin tests is subject to considerable variation in the same individual.[10] A total or consistent inhibition of the reactions by ACTH would have been of striking significance had this occurred, but it did so in only one case. The passive transfer phenomenon showed diminution in 2 and increase in 3 instances following ACTH therapy (Chart 5). The others remained essentially the same as before treatment. There is, moreover, no correlation between diminution of the scratch or passive transfer tests and a rise in 17-ketosteroids. We may conclude, therefore, that ACTH administered in the dosage and time interval outlined previously has no appreciable effect on direct or passive transfer skin tests in hay fever patients, in spite of the alleviation of their hay fever symptoms. One may postulate further that ACTH, through the adrenal hormones, does not interfere with the antigen-antibody reaction in the immediate type of hypersensitivity, but rather that it exerts its blocking effect at some farther end point. Continued administration of ACTH for a longer period and in larger dosage, however, may further alter the immediate type of skin reaction.

SUMMARY

1. Ten hay fever patients were given 20 mg. of ACTH intramuscularly every 6 hours for 8 doses.

2. Discomfort of hay fever in the 4 patients with active symptoms disappeared temporarily.

3. ACTH in the dosage and time interval outlined did not significantly alter the positive scratch or passive transfer skin tests.

4. Adrenal response to ACTH was demonstrated by a fall in circulating eosinophils in all of the 10 patients and by a rise in urinary 17-ketosteroids in 6 cases.

The ACTH used in this study was obtained from the Armour Laboratories through the kindness of Dr. J. R. Mote.

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THE RELATIONSHIP OF THE ETIOLOGIC FACTORS IN ASTHMA IN INFANTS AND CHILDREN*

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THE relative importance of foods, inhalants, and infection in the etiology of asthma in infants and children has long been the subject of much controversy. That foods cause asthma in sensitive individuals is undisputed, but there is no agreement, however, concerning the frequency with which this occurs. It has long been held that food sensitivity is the principal cause of asthma in infants and children. Statements to this effect are found in almost every textbook on allergy as well as in numerous articles published on the subject of asthma in children. All investigators do not agree and opinions vary from that of Rowe and others, who insist that foods are the major factor, to those who believe that asthma from food alone is uncommon in infants and young children.

For example, Rowe and Rowe[1] reported that food sensitivity was the sole cause of asthma in 50 per cent of 411 children under the age of 5. Stoesser,[2] in a study of 329 children with asthma, found food allergens to be very important in fancy and early childhood, while the inhalants were most significant in the latter part of the preschool period. The same opinion was expressed by Hill,[3] who found 24 per cent of 100 children 3 to 12 years of age who had asthmatic attacks following the ingestion of certain foods. Tuft[4] stated, "It would seem that foods are probably the most important group of allergens in infants and young children."

The most recent literature on asthma in children has, on the whole, been more conservative in its opinion as to the importance of foods in asthma. Spain and Cooke[5] felt that while food is undeniably a cause of asthma, it has been assigned too much importance by many physicians. Ratner,[6] in a recent article, noted that although it has been thought that food sensitivities play the dominant role in allergy in childhood, his experiences differed, for throughout infancy and childhood sensitivities to foods, inhalants, and contactants run a more or less parallel course. A good deal of the confusion regarding the frequency and importance of clinical food sensitivity is due to the methods used in arriving at a diagnosis. Skin tests alone, either scratch or intradermal, do not confirm the presence of respiratory allergy to foods. Elimination diets have been of considerable value. The method we have used consists of (1) intradermal skin testing to a representative group of 36 common foods; and (2) provocative feeding on 3 separate occasions with those foods found positive on test and those which gave a positive clinical history with negative skin tests. By these simple procedures we feel that the majority of the true food sensitivities can be ferreted out. Elimination diets as described by Rowe are used in those cases where direct tests and the history are inadequate.

*From the Department of Pediatrics, The University Hospital, New York University Bellevue Medical Center.

There is general agreement as to the incidence of the etiologic factors concerned in the diagnosis of inhalant respiratory allergy. Pollens, fungi, house dust, animal dander, and others are commonly recognized causes of asthma in children. The incidence with which inhalant factors occur varies slightly with the observer. It is in the field of foods as a diagnostic factor that the most marked differences of opinion occur.

The third cause of asthma in infancy and childhood is, in our opinion, chronic upper respiratory infection. These cases are most commonly termed infective asthmatics.

The incidence and importance of infective asthma in infants and children are, however, the subject of a very considerable difference of opinion. Standard texts differ markedly. Feinberg[7] said, "Infective asthma in the child is the exception rather than the rule." Tuft[8] stated, "Focal infection in the tonsils and teeth rarely causes asthma in children." On the other hand, Cooke[9] and Chobot[10] have emphasized the importance of bacterial allergy. The primary argument of those who do not believe in the existence of bacterial allergy or infective asthma is the inability to obtain positive skin tests to bacteria or to demonstrate the presence of skin-sensitizing antibodies circulating in the blood of these patients. Yet it is freely admitted that clinical food sensitivity may exist in the absence of both positive skin tests and skin-sensitizing antibodies circulating in the blood of these patients. To us it seems rather difficult to corroborate the existence of food allergy without a demonstrable immunologic mechanism and then to rule out the possibility of the existence of bacterial allergy because no mechanism is demonstrable. For clarity and because the diagnostic standards for bacterial allergy are not universally accepted we have outlined criteria for the diagnosis of bacterial hypersensitivity. These have been stated by Cooke[11] and slightly modified by us:

1. Past personal and family history of allergy
2. Repeated respiratory infections associated with or followed promptly by asthma
3. A typical asthmatic chest without parenchymatous involvement of the lung
4. Eosinophilia (the higher eosinophil counts are more likely to be found in the infective, rather than food or inhalant type of asthma)
5. Leucocytosis and increased sedimentation rate may or may not be present
6. Results of therapy based on infection as the causative factor
7. Improvement promptly ensues on the removal of an infected focus in properly selected cases
8. Isolation of pure cultures of pathogenic organisms from suspicious foci of infection
9. The ability to produce an attack of asthma with a given dose of autogenous vaccine

The impression that food allergy was overemphasized as a cause of asthma in infancy, together with the denial of the existence of bacterial allergy by many pediatricians and allergists, prompted this study of the causes of asthma in infancy.

PROCEDURE

Four hundred children whose asthma began before they were 3 years of age were studied. Records were taken from the Pediatric Allergy Clinic of the University Hospital and from our private practices. Thorough history and physical examination, otolaryngological survey, hemograms, urinalyses, Mantoux tests, and roentgenograms of chest and sinuses were done. Intradermal tests of the usual inhalants and 36 common foods were performed.

Clinical food trials were performed as previously outlined: elimination of inhalant factors was done where possible, and desensitization with pollen and house dust was instituted; repeated interviews and progress reports were noted. These children have now been under our care for varying periods of time, from a few months to 12 years. The assembled data are presented in Tables I and II.

TABLE I. CAUSES OF ASTHMA IN 400 INFANTS AND CHILDREN WHOSE ASTHMA BEGAN BEFORE AGE OF 3 YEARS

FACTORS	PER CENT
Sole factors	
Infection	30.50
Inhalant	10.25
Food	0.25
Total	41.00
Combined factors	
Infection, inhalant	44.25
Infection, food	4.75
Infection, inhalant, food	7.75
Inhalant, food	2.25
Total	59.00

RESULTS

We have analyzed our cases and divided them into 2 groups: (1) those having a single etiologic factor, that is, infection, inhalant, or food, and (2) those with a combination of these factors, such as inhalant and infection, inhalant and food, infection and food, or all three.

TABLE II.

Infection (alone and combined)	
Infection	30.50
Infection + inhalant	44.25
Infection + food	4.75
Infection + inhalant + food	7.75
Total	87.25%
Inhalant (alone and combined)	
Inhalant	10.25
Inhalant + infection	44.25
Inhalant + food	2.25
Inhalant + infection + food	7.75
Total	64.5%
Food (alone and combined)	
Food	0.25
Food + infection	4.75
Food + inhalant	2.25
Food + inhalant + infection	7.75
Total	15.0%

We determined a single cause of asthma in 41 per cent of our cases. Surprising as it may seem, only 1 child of the 400 studied had asthma resulting from a food without other causative factors. Inhalants caused 10 per cent of the cases, and infection 30 per cent.

Multiple factors caused asthma in 59 per cent of the children. Foods were considered etiologically significant, usually in a secondary role, in 15 per cent of all the cases; inhalant factors, usually in combination with infection, occurred in 65 per cent, while infection associated with the other 2 factors composed the largest group, totaling 87 per cent. Examination of the tables will indicate this in further detail.

Upper respiratory infection is first in importance as a cause of asthma in this age group. Inhalant sensitivities and foods follow in importance. Inhalants may be important in the early years, contrary to previously expressed opinions. These findings indicate that food allergy has been overemphasized as a cause of asthma in infancy. At the same time infection as a cause of asthma in infancy has been underestimated.

Bacterial allergy is of the greatest importance in these children and may simulate any allergic syndrome. Chronic focal infection as a cause of asthma must be recognized as an entity and therapy directed toward its control. The fact that it was the sole cause of asthma in 30 per cent of these cases, where combined with inhalant sensitivity accounted for 44 per cent, and where combined with food or with both other sensitivities totaled 13 per cent, indicates that in 87 per cent of the cases infection was a factor. It is true that better and more sensitive methods of diagnosing bacterial hypersensitivity must be developed. Despite this however, it must be acknowledged that infective asthma exists in infancy and is one of the most important clinical manifestations of bacterial allergy.

Thus, in order of importance, infection ranks first, inhalant sensitivity second, and food third. These findings are important in our opinion. Our figures would indicate the incidence of food allergy in asthma to have been grossly overestimated. Conversely the role of infection in asthma has been sadly underestimated. The inhalant sensitivities are important factors in the early years, and appear at an earlier age than has usually been described.

SUMMARY

1. Four hundred infants and children whose asthma began before the age of 3 have been studied to determine the relative importance of foods, inhalants, and infections.

2. Foods play a subordinate role to inhalants and infections in childhood asthma.

3. Inhalant factors are more important than previously considered in asthma in infants.

4. Chronic focal infection is one of the most important causes of asthma in infants and children.

5. Multiple causes of asthma were found in 59 per cent of the cases, single causes in 41 per cent.

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INTERSTITIAL MEDIASTINAL EMPHYSEMA DUE TO BRONCHIAL ASTHMA

Case Report

I. S. Kahn, M.D., and J. W. H. Rouse, M.D., San Antonio, Texas

INTERSTITIAL mediastinal emphysema complicating asthma is extremely rare. Of the 73 cases[1, 2] of spontaneous mediastinal emphysema reported in the literature, only 28 were due to asthma. There has been but one fatal case reported and the pathologic findings in this case did not demonstrate clearly the cause or the point of rupture of the lung.[2] It may be of interest that the first case reported in American literature was presented by this office in 1927.[3] This patient fully recovered from her pulmonary accident and was successfully treated for her asthma. She was seen 20 years later still free of asthma and had had no recurrence of her emphysema.

CASE REPORT

A 5-year-old boy, for the previous five months, had been under desensitization for asthma in our office with satisfactory results. During the period of desensitization he had several recurrent attacks of mild asthma, all of which responded to small doses of epinephrine. These attacks had been less frequent and progressively milder up to the time of his pulmonary accident. A few days prior to Nov. 3, 1949, the family moved into a comparatively old house in a different part of town. On Nov. 3, the mother reported by phone that the child had developed mild asthma. We felt that the recurrence of asthma was probably due to excessive exposure to house dust incident to their moving, and we instructed the mother to use 3 minim doses of epinephrine 1:1000 every 2 hours for 3 doses. Because of strenuous objections on the part of the child, the mother did not follow our instructions and allowed the child to cough and wheeze during the night. The next morning the mother noticed a distinct swelling about the neck and face. She immediately notified her pediatrician and the child was subsequently hospitalized.

On examination in the hospital, the child was found to be moderately dyspneic, nervous, and apprehensive. There was considerable bilateral swelling about the neck and the anterior thoracic wall, the emphysema extending slightly into the cheeks bilaterally. The respiratory excursion was retarded on the left side suggesting pneumothorax. This, however, was not borne out by x-ray. Palpation showed the usual crepitation incident to subcutaneous emphysema, and auscultation over the involved area revealed a typical crunching sound as if Cellophane were being wrinkled. The respiratory rate was 36, pulse about 150, and temperature normal. There was x-ray evidence of widespread mediastinal emphysema, emphysema about the pericardium, and some tracheal compression. There was no pneumothorax or atelectasis. Pain over the chest was absent. According to the mother, the boy had never complained of pain during the illness. The child was put at complete rest and the only medication was epinephrine 3 minims subcutaneously given at 3 hour intervals. The swelling and crepitation began to clear after about 12 hours, progressively improving until both were gone in 5 days. Asthmatic symptoms, which were very minimal, were controlled from the beginning, and at the end of 24 hours epinephrine was discontinued.

In considering the possible explanation of the sequence of pathologic changes in this case, we believe that the following explanation is logical. We know that

bronchial smooth muscle extends down to the atrium of the alveoli. Therefore muscular fiber constriction could have closed the small bronchialis leading into an alveoli sac. This would create a condition similar to that which exists when a toy balloon is inflated with air, and constricted at its opening. If the lung were then compressed by contracting the diaphragm during an asthmatic cough, rupture of an alveolar sac might easily result, particularly if the epithelial wall of the alveolar sac had been weakened by intracellular edema. Following rupture, air may then travel along the perivascular sheaths as this is the path of least resistance. During respiratory movement, there is a shortening and

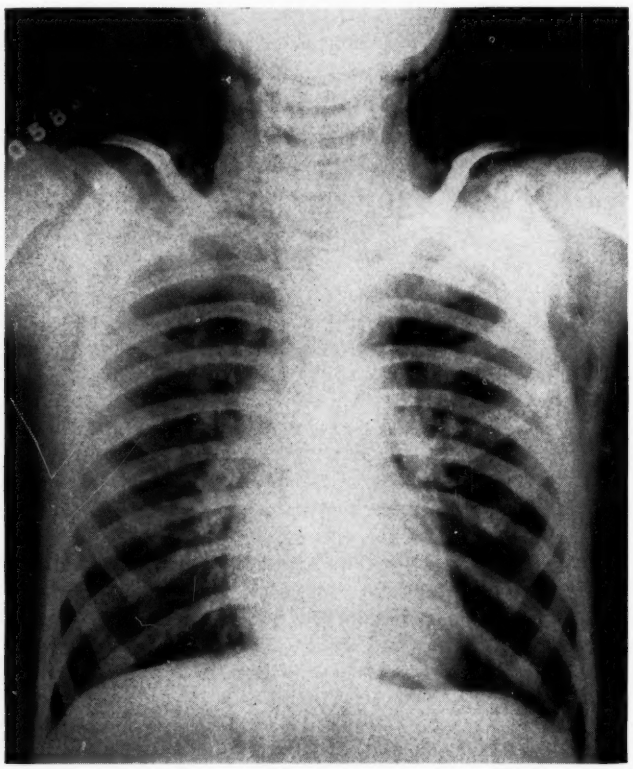

Fig. 1.—Roentgenogram showing widespread mediastinal emphysema, emphysema extending into the neck and face, emphysema about the pericardium, and some tracheal compression.

lengthening of blood vessels, by which action escaped air could be moved on up the course of the vessel to the hylus of the lung. From there air would move to the post and middle mediastinum, then forward between the parietal pleura to the pericardium, then to the anterior mediastinum, neck, and thorax. The amount of compression from the air on the great vessels, heart, and surrounding structures usually produces pain, the radiation of which would depend upon the areas involved. Our patient did not develop pain. Therefore, we feel that soft tissue gave way sufficiently, and there was no great amount of compression or separation of tissue. It has been postulated that emphysematous

blebs may develop along the pleural surfaces. These blebs may rupture, producing similar pathologic changes or pneumothorax. We feel, however, that the preceding explanation is the most logical in this case.

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VASCULAR ALLERGY: EXPERIMENTAL STUDIES ON ALLERGIC THROMBOSIS OF THE RETINAL VEINS*

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SEVERAL significant clinical and experimental observations have been made on the relation between allergy and vascular diseases. To mention only the principal vascular conditions, we may recall that many investigators believe that sensitivity to tobacco rather than toxicity is responsible for many cases of thromboangiitis obliterans. Harkavy[1] reported positive skin reactions to tobacco in patients with Buerger's disease.

Periarteritis nodosa also is believed to be the result of an allergic reaction provoked by various substances such as bacterial, drug, serum, and possibly food or inhalant allergens. Rich[2] reported the finding of acute arterial lesions, similar to those of periarteritis nodosa, in patients who received both heterologous serum and sulfonamides. Experimentally, Rich and Gregory[3] showed arterial lesions varying from simple edema to advanced medial necrosis and cellular infiltration in serum-treated rabbits. It has been suggested that the temporal arteritis is due to hypersensitivity. The relationship to allergy is not definite, but it may represent the highest possible degree of localization of an arterial lesion (Miale[4]). According to Harkavy's studies angina pectoris also may be due sometimes to tobacco allergy.

As regards thrombophlebitis, it is worth remembering that in many instances cultures of the veins and thrombi do not themselves show the presence of organisms. This may be an argument in favor of an allergic etiology. On the other hand, it is known that thrombophlebitis is seen often in association with erythema nodosum, asthma, tuberculosis, and many other inflammatory processes. It has been postulated that this type of thrombophlebitis represents a reaction of a sensitive tissue to a toxin (Allen, Barker and Hines[5]). Harkavy found a positive skin reaction to extracts of tobacco in cases of recurrent idiopathic thrombophlebitis.

Finally, we must remember that very few attempts have been made to demonstrate the probable existence of a venous thrombosis of allergic origin. Therefore we have undertaken these experiments with the purpose of investigating the possibility of eliciting a venous thrombosis with an allergic mechanism. Since it is possible to study ophthalmoscopically the pathologic manifestations of the fundus, we preferred to undertake our investigations on the retinal veins.

METHODS

We followed the methods that one of us (P. D. M.[8]) used in his experimental studies on allergic cholecystitis. The studies were performed upon 15 rabbits of nearly the same age and weight. The animals were sensitized by 5

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intravenous injections of 2 ml. of sheep serum at intervals of 5 days. Ten to 12 days after the last sensitizing injection, the exciting dose (0.10 ml. of sterile sheep serum) was inoculated into the vitreum.

The eyes of the animals were examined before and after the exciting dose by the ophthalmoscope and the slit-lamp.

The fundus examinations began 1 hour after the exciting dose and continued every 4 to 5 hours in the first 2 days, and afterward twice a day. The rabbits were killed 1, 3, 6, and 24 hours after the exciting dose and the eyes enucleated. Three animals were sacrificed 15 to 20 days after the exciting inoculation.

Control tests were made in 7 nonsensitized rabbits which received in the vitreum 0.10 ml. of sterile sheep serum. After the animals were killed, the eyes were enucleated and fixed in 10 per cent neutral formalin.

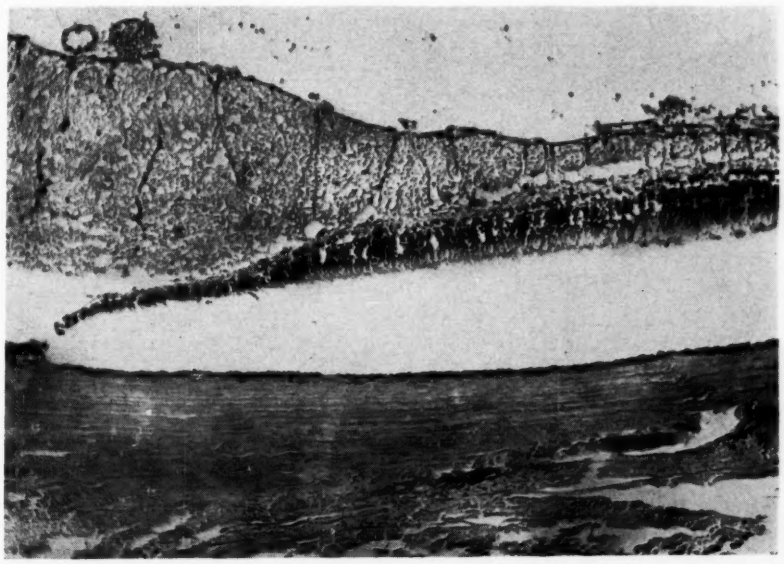

Fig. 1.—A thrombosed branch of the central vein (×90).

RESULTS

We shall omit discussion of the allergic reaction of the uveal tract, since this has already been observed by several investigators. We found more interesting the retinal manifestations.

Ophthalmoscopic examination 1 and 3 hours after the exciting inoculation revealed vasal dilatation and congestion. Some of the retinal veins contained an amorphous and acidophilous substance.

At 6 and 24 hours after the exciting inoculation, the fundus picture was that of a thrombosis of the retinal veins. Microscopically, the central vein and its branches were thrombosed (Figs. 1 and 2). The characteristics of the thrombus were those of the white thrombus.

It was not possible to study the pathologic changes of the vein wall because the thrombus was in such close adherence to the wall. In some animals the thrombosis involved a large portion of the vein (Fig. 3).

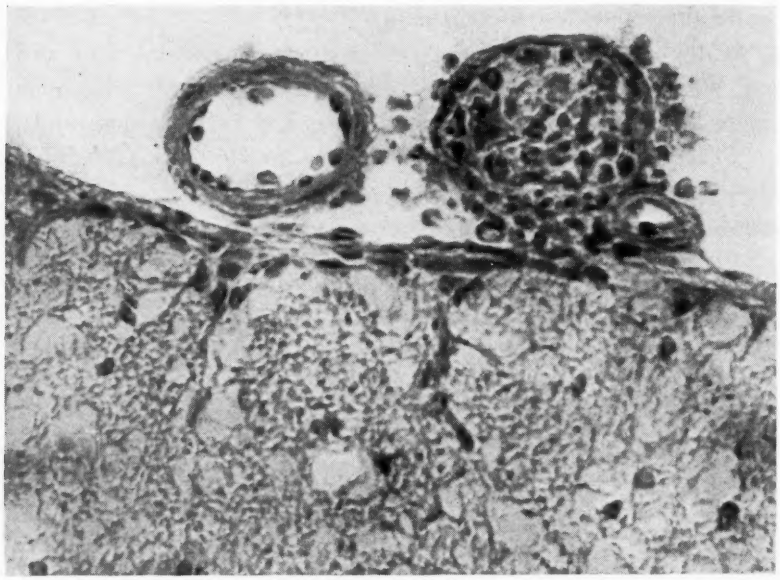

Fig. 2.—High magnification of Fig. 1 (×380).

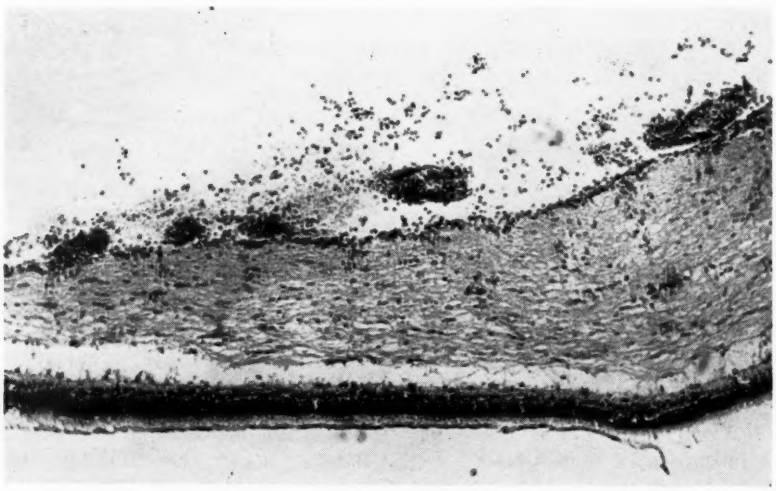

Fig. 3.—Longitudinal section of a thrombosed retinal vein (×90).

The hemorrhages, which followed the thrombotic process, were found in the inner layers of the retina and in the nervous fibers of the optic papilla (Fig. 4), or in the preretinal zone (Fig. 5), and sometimes in the vitreum.

Besides these pathologic changes, others were observed. The vitreum showed cellular infiltration, including numerous eosinophils. The optic papilla was edematous.

It is worth noting that the choroid was only slightly hyperemic.

In the animals killed 15 to 20 days after the exciting inoculation the retinal veins were still thrombosed and there was a well-developed organization of the thrombus. In some veins the obstruction was partial; in some others the thrombus completely blocked the vessel.

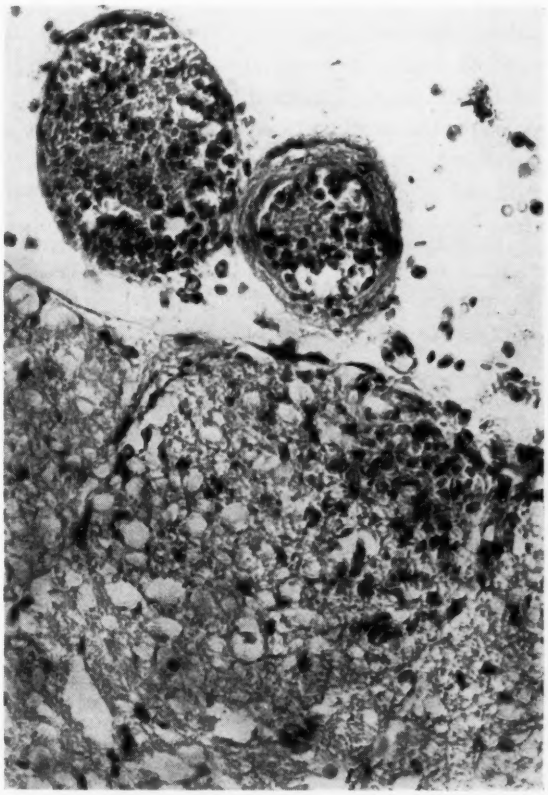

Fig. 4.—Thrombosis of the central vein, with hemorrhages in the nerve fibers of the optic papilla (×380).

When the retinal veins were completely occluded, the retina was almost entirely destroyed and the choroid showed a marked histiocytic reaction. Also, in the vitreum a more or less marked cellular infiltration was sometimes evident, including numerous histiocytes.

In the optic papilla there were new formations of blood vessels and a definite infiltration by histiocytes.

In the nonsensitized control animals no anatomic changes were found except in 2 rabbits, in which a slight iridocyclitis caused by the inoculation of a heterogeneous substance was observed.

DISCUSSION

The results of our experimental studies demonstrate the development of an allergic thrombosis of the retinal veins, while the control studies (in non-sensitized animals) were practically negative.

The thrombus, as we said previously, was made up almost entirely of platelets, and there was also a certain admixture of fibrin and leucocytes.

No published reports are known to us on the allergic thrombosis of the retinal veins. Stewart Duke-Elder,[6] in his textbook of ophthalmology, pointed out that 2 distinct factors enter into the etiology of the thrombosis of the retinal veins: general vascular sclerosis and toxic factors. Bedell and Bothman[7] observed the occurrence of retinal hemorrhages following tetanus antitoxin injections. Two cases of retinal hemorrhages due to food allergy were mentioned by Wittich. Bienstock reported scotomata and spasms of the retinal blood vessels in himself. He was able to prevent the appearance of these symptoms by elimination of certain animal proteins from his diet, and reproduce them at will by ingestion of these foods.

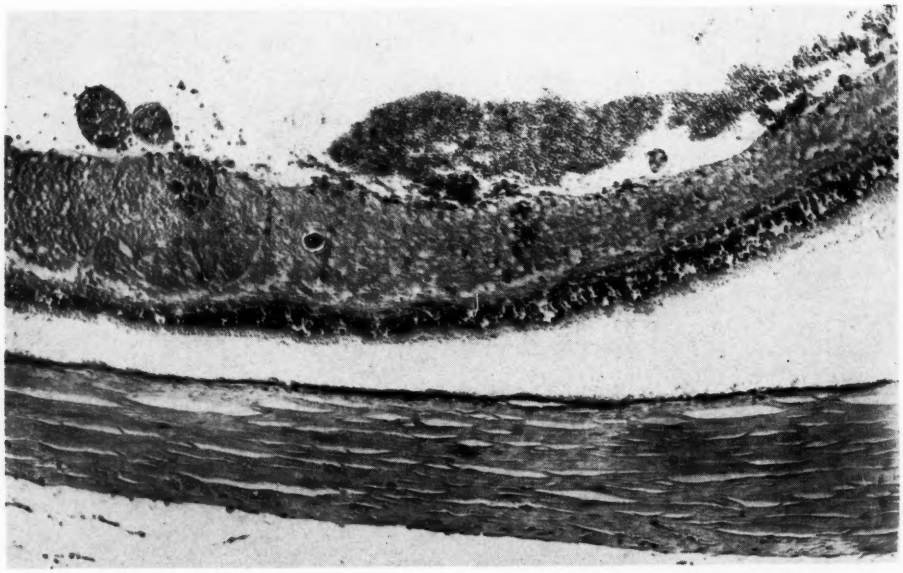

Fig. 5.—Thrombosis of the central vein and hemorrhages in the preretinal zone (×90).

Our experimental studies substantiate the possibility of allergic reactions of the retinal vessels and may allow us to explain some cases of thrombosis of the retinal veins which occur suddenly in healthy young people.

Applying the same methods to other parts of the venous system it seems possible that one might be able to provoke a thrombus formation similar to that produced in the retinal veins.

It is difficult to say by which mechanism the thrombosis has been produced in our animals. It seems reasonable to suppose that the inflammation of the

vessel wall may be the predominant factor. Platelets and leucocytes would adhere to the roughened endothelium and clotting mechanism would commence.

Further experiments are necessary to confirm this hypothesis.

SUMMARY

The aforementioned experiments were performed upon 15 rabbits sensitized by 5 intravenous injections of 2 ml. of sheep serum. The exciting dose was inoculated into the vitreum. At 6 and 24 hours after the exciting inoculation a thrombosis of the retinal veins was observed. In the animals killed 15 to 20 days after the exciting dose the vessels were still thrombosed and there was a well-developed organization of the thrombus.

In the control studies on nonsensitized animals, the inoculation of the sterile sheep serum did not cause any pathologic changes.

The mechanism of the thrombus formation is discussed.

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ALLERGENIC FRACTIONS OF LOW RAGWEED POLLEN. II. SOME IMMUNOLOGIC, ELECTROPHORETIC, AND CHEMICAL CHARACTERISTICS OF DIFFUSATES*†

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THERE has been considerable speculation as to the molecular size of pollen excitants. Abundant confirmation was found for the early claim of Grove and Coca[6] that the activity did not diffuse to any important degree through cellophane. Of the opposite opinion were Unger, Cromwell and Moore,[21] Johnson and Rappaport,[7] Spain and Newell,[17] Benjamins, Dishoeck, and German,[3] Long and Teller,[8] and, more recently, Robbins, Samuels, and Mosko,[14] Stone, Harkavy, and Brooks,[19] Cohen and Friedman,[4] and Stevens and Moore.[18] Stull, Sherman, and Wing[20] found activity in both diffusible and nondiffusible fractions prepared by salting-out, a situation also encountered by the writer during ultrafiltration studies.[11] From the latter 2 laboratories came the additional observation that the susceptibility for smaller substances varied with the patient, some failing to respond altogether, whereas the larger molecules were allergenic without exception. That this selectivity rested on specificity differences was demonstrated by serologic cross-tests.

Abramson, Moore, and Gettner[1] concluded from ultracentrifugal and diffusion studies that the major allergen of undialyzed ragweed extract had a molecular weight of 5,000, was unpigmented and essentially immobile in the electric field (electrophoresis). Rockwell,[15] using chemical methods instead of electrophoresis for his separations, agreed with Abramson and colleagues as to molecular size. Sanigar,[16] however, noted marked heterogeneity in the ultracentrifuge and could only conclude that most of the pollen material was below 17,000 in molecular weight. Newell,[13] attempting to rid the extract of the low-molecular excitant of Abramson, subjected it to 20 hour dialysis through cellophane before analyzing it electrophoretically. The pattern, nevertheless, resembled that of Abramson's undialyzed preparation. When Newell examined in this apparatus the 3 fractions salted out by Stull, one of which ("Fraction 2") was dialyzable while the others were not, he obtained for each the familiar pattern—a major, immobile, colorless constituent, and a minor, mobile, pigmented group. Thus, a dilemma arose as to the nature of Abramson's major component, since he reported it to be dialyzable, whereas Newell was unable to eliminate it through 20 hour dialysis and found a similar component in all of Stull's fractions.

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Differences in the immunizing potentialities of the diffusible and nondiffusible portions of low ragweed pollen have been examined by the Roosevelt Hospital group[20] and the writer.[11] In both studies it was found that the nondiffusible material was far more efficient in producing blocking antibodies and clinical resistance than was the dialyzable material. Finally, chromatographic separations have revealed[11] that the ultrafiltrate contains several active pigments of different specificity.

The situation to date could be summarized as follows: Small dialyzable constituents in ragweed pollen differ in specificity from nondiffusible components. They can provoke clinical allergy in most, but not all, patients with a history of autumnal hay fever who are reactive to whole extract. They are ineffectual in developing clinical resistance and thermostable (blocking) antibodies, behaving like haptens. To date, no evidence has been published to show that the diffusible and nondiffusible portions of the extract differ from each other electrophoretically. Such evidence will be presented in this article. From the chemical viewpoint, Abramson considered his major fraction to be protein-like although it contained carbohydrate. Likewise, Stull decided that the nitrogenous portion of his dialyzable fraction 2 was the important immunologic substance, albeit some samples gave a positive Benedict's test. The author found both precipitable nitrogen and Molisch-positive constituents in her ultrafiltrates.

The relationships between molecular size and the chemical, electrophoretic, and allergenic nature of ragweed pollen antigens are, therefore, far from clear. This is due to the complexity of the problem, which has induced many workers to concentrate on the separatory phase at the expense of immunologic and other tests for purity. It was the purpose of the present investigation to converge the 2 aspects. Pollen dialysate, for example, has been visualized for the first time in the Tiselius apparatus. Preliminary electrophoretic comparisons have been made between the diffusate, the dialyzed sac contents, and whole extract. In addition, the dialysate has been separated into fractions by means of electrophoresis as well as by chromatography, and specificity differences have been shown to exist between them by means of immunologic cross tests. Not only has the significance of the small molecular material for the patient been extensively appraised by direct sensitivity tests, but its immunizing potentialities have been re-explored by therapeutic injections given man. Its stability has been observed under freeze-drying and under heat. The nature and degree of the loss of material and activity from the extract during dialysis have been investigated under controlled conditions for the first time.

MATERIAL AND METHODS

All extraction and separatory steps were carried out in the 4° C. coldroom with precautions against contamination by molds and bacteria. Periodic tests for sterility allowed the prompt interruption by filtration of any contamination.

Allergenic Solutions.—With 2 exceptions all pollen had been collected under the "gold seal" precautions of Hodgson during the autumns of 1947 and 1948. Whole extracts were made by exposing approximately 20 Gm. of ether-defatted, low ragweed pollen (*Ambrosia elatior* or *A. artemisiifolia*)

to 100 ml. of phosphate buffer of .06 ionic strength at pH 7.4 for 2 hours with mechanical stirring, or for 42 hours without stirring. In 1 large set of conjunctival comparisons between dialysate and our "standard" whole extract, the preparation differed from other whole extracts only in that alkaline-saline solution had been used as extractant. Each extract was sterilized by passage through bacterial filters of the Seitz or sintered glass variety, and labelled according to the date of its analysis and its content of phosphotungstic-acid precipitable nitrogen, 0.1 mg. representing 10,000 PTA-N units.

Dialysates and filtrates were prepared from whole extracts in 2 ways. The first was the conventional procedure of placing a measure of extract inside a Visking cellophane (sausage casing) sac against 1 to 3 parts of either distilled water or phosphate buffer for a period ranging from 16 to 87 hours. The other was the application of gentle suction with a water filter pump under aseptic conditions to a cellophane sac placed within a dry sterile suction flask, until most of the fluid had been drawn out of the sac during 17 days. The product was labelled "vacuum dialysate" (or "filtrate"). In 1 instance a secondary dialysate was made by re-exposing the sac contents to a different buffer; namely, whole extract of Jan. 31, 1949, was first exposed to 2 volumes of phosphate buffer for 18 hours and subsequently to 3½ volumes of barbital buffer, ionic strength 0.1, pH 7.9, for 46 hours.

In most of the earlier studies, once an initial diffusate had been prepared for testing, the balance of the voluminous dialysates was discarded. In an effort to rid the sac contents of all diffusible molecules, running water had been employed for about 5 days and electrodialysis for 72 hours. So striking were the losses of material and activity from the sac contents during these prolonged dialyses that it was decided to modify one set of experiments by saving aliquots of dialysate at each stage in order to see whether these losses were referable to a mere shift of materials or to degradation processes. Thus, in Experiment XVII 4 identical preparations of extract were exposed to 1 or 1½ parts of distilled water for 2½ days, to provide initial dialysate. Next, 36 L. of distilled water were allowed slowly to pass the sac under a hydrostatic head of about 2 feet of water which maintained continuous negative pressure outside the sac. (This secondary diffusate was referred to as "hydrostatic" dialysate.) Finally, electrodialysis was carried out on the sac contents by surrounding the sac with 16 L. of continuously circulating distilled water which was changed 3 times during 96 hours. Meantime, a current of 27 Ma., which gradually fell to 6 Ma., was used at 105 volts. Samples were removed from the various diffusates and sac contents at each stage so that activity and chemical studies could be done. Aliquots of the highly diluted dialysates were dried in the frozen state so that they could be reconstituted later in small volumes.

Stability of whole extract XIV, whole standard extract, and vacuum dialysate was achieved by addition of an equal volume of glycerine, whereas the sac contents of Experiment XVII, like the dialysates, were kept in the lyophilized state. Whole extract XII and sac contents XI, like the first and second dialysates of Jan. 31, 1949, were unpreserved but were tested within a few months of their preparation.

Chemistry.—All fractions, with the exception of the small yields from the electrophoretic apparatus, were analyzed for PTA-precipitable nitrogen and nonprecipitable nitrogen by the micro-Kjeldahl method, as well as for combined and free reducing sugars by a slightly modified Folin-Malmros procedure.[5] Assays on secondary dialysates were considered less reliable than on the other solutions, due to their small content of solids. The aim of the semiquantitative analyses was to establish roughly the chemical nature of our incompletely purified fractions and to determine whether simple diffusion or enzymatic breakdown was responsible for the loss of activity from the dialyzing sac.

Activity Tests.—Potency was judged by means of serologic, conjunctival, and intracutaneous tests carried out in a controlled manner.[9] Although our original intention was only to determine, by any of these methods, the approximate dose of our several derivatives which would provoke comparable responses in a given patient, we subsequently attempted to gain an over-all impression of each fraction by grouping the combined results of the 3 techniques under 5 headings, namely, +++, ++, +, ±, and 0.

In preliminary Prausnitz-Küstner experiments, we found that +++ responses could be elicited with one-third of the serum specimens when 40 unit unglycerinated whole extract or sac contents was selected for the test. To procure this proportion of marked reactions with dialysate, however, it was necessary to use 160 unit material. The serum of each patient was accordingly examined with 0.03 ml. of these 2 strengths, and the potency of the 3 unglycerinated preparations was judged by the relative number of ++, +, and negative responses each provoked.

Whereas a single test with 0.03 ml. of 160 unit unglycerinated dialysate served for the crude classification of patients during several months by intracutaneous test, more careful examinations were carried out with glycerinated whole extract by means of serial strengths which located the minimal reactive dose. In order to include these results in the composite analysis, the threshold values were later assorted into 5 classes, based on the response to 0.03 ml. of 1 unit whole extract, a yardstick which placed a little over one-third of the patients in the +++ class. The need to select so dilute a test material confirms the observations that the intracutaneous test is more sensitive than is the Prausnitz-Küstner method and that glycerine preserves pollen activity.

The conjunctival test was also carried out in such a manner as to indicate the thresholds of susceptibility for unfractionated and derivative antigens. The results were again sorted roughly into 5 groups according to the thresholds. As in the case of the Prausnitz-Küstner results, a fourfold handicap had to be allowed dialysate.

In addition to estimating the capacity of dialysate to combine with preformed reagins by the preceding means, we also recorded any systemic manifestations which appeared following the therapeutic use of vacuum dialysate in 8 patients.

Dialysate and sac contents were heated in a water bath at 60° C. and 100° C. for 1 hour and then examined serologically for potency. The effect of freeze-drying was likewise examined by this technique. Specificity differences in the

various dialysates, sac contents, and starting materials were examined by means of cross-neutralization studies carried out in normal test subjects. Twenty-four hours after a series of intracutaneous injections had been made with sensitizing serum to which a pollen fraction had been added in excess, the sites were tested with the homologous allergen and with other fractions. Whereas in vitro neutralization of the homologous allergen had usually occurred, as evidenced by the lack of response to its reintroduction in high concentration on the following day, fractions which differed from it in specificity could still elicit a wheal-and-flare response in high dilution. The amount of PTA-nitrogen required to provoke this cross-reaction was then compared with the amount needed to reproduce the urticarial result in a site which had received only serum and saline solution. The nonirritating quality of the antigen was established by its concurrent use in high concentration in areas of normal skin. By comparing these 3 reactions, it was possible to classify the specific cross response as being maximal, half-maximal, ±, or negative.

Therapeutic Procedure.—Eight dialysate-allergic patients were given a single therapeutic injection of vacuum dialysate XIV in a dose ranging from 1,600 PTA units to 3,000 units. Just before use, the undiluted filtrate containing .77 mg. PTA-nitrogen per c.c. was mixed with 6.7 parts of the patient's own serum, in the hope that specific spontaneous conjugation would take place and render this haptenlike preparation more antigenic as far as immunization was concerned. In 2 control cases saline solution was substituted for the patient's serum. The dialysate was then stirred with mineral oil and Falba to make a stable, water-in-oil emulsion which was then placed in a subcutaneous depot, an abbreviated immunzation procedure which the author has found effective for whole pollen extract.[10] All but 2 of the subjects also received whole extract or sac contents in another such depot. The immunologic response to this complementary therapy was followed by monthly eye tests, and such clinical resistance as developed was compared with that of past seasons when depot therapy with whole ragweed extract alone had been involved.

The antibody response to whole extract, with its dialyzable constituents intact, was judged by monthly eye tests with both vacuum dialysate and whole extract on 84 patients who had been treated with unfractionated extract in emulsified state.

Electrophoretic Studies.—Patterns were obtained on samples of whole extract XII, electrodialyzed sac contents XI, and first dialysate. Heretofore, characterization of dialysates of pollen has not been accomplished, presumably because of their ready dialyzability, which complicates the interpretation and leads to marked loss of material from the sample during its equilibration against a large volume of the buffer to be used in the run. This loss was discouraged by the use of 7 thicknesses of cellophane and by restricting the period of equilibration to 3 hours. The result was only partially satisfactory, since the sample was brought to a resistance of 300 ohms, whereas the buffer registered 467 ohms. The possible influence of this disequilibration on the pattern will be discussed. Whole extract and sac contents were equilibrated by exposure to 250 volumes of phosphate buffer for 19 and 67 hours, respectively.

Whereas the unfractionated extract and electrodialyzed material were analyzed in their original concentrations, first dialysate had been reduced to dryness in the frozen state and taken up in one-fifth its original volume of distilled water so as to increase the concentration of its solids. Preceding its exposure to the Tiselius buffer, this dialysate was protected by 3 thicknesses of cellophane and suspended in 9 volumes of distilled water for 30 minutes in order to lower its phosphate salt content.

In all electrophoretic runs phosphate buffer at pH 7.5 and ionic strength 0.1 was employed with a current of 13.5 Ma. at 175 to 200 volts. The experiment was interrupted after 135 minutes in the case of whole extract and after 153 minutes in the case of sac contents, whereas first dialysate was observed for 357 minutes to see whether its major stationary component would move. Mobilities were not calculated due to the low peaks of the pattern of the unconcentrated samples and the disequilibration of the dialysate, but the relative areas of mobile and immobile components were estimated for each sample.

At the conclusion of the runs on sac contents and dialysate, a small sample of the fastest- and of the slowest-moving material was removed from the cell for immunologic cross-testing.

Chromatography.—Several hundred c.c. of the distilled water dialysates, XVII No. 7, No. 8 and No. 9, were adjusted to pH 5 as in earlier absorptions,[11] and each was allowed to seep through 3 to 5 Gooch crucible holders which had been packed with mixtures of alumina and Supercel and connected in vertical series. The 3 yellow bands of adsorbed material were dug out and eluted in phosphate buffer at pH 8. Only a small portion of the adsorbed pigment was removable. Eluates were nevertheless analyzed for specificity differences and potency by the serologic technique.

FINDINGS

Chemistry.—The nitrogen and carbohydrate contents of the extract dwindled as dialysis proceeded. The dialysates procured by allowing 1 or 1½ volumes of distilled water to stand against whole extract for 2 to 3 days robbed the cellophane sac of two-thirds of its nonprecipitable nitrogen, one-half its reducing sugars, one-fourth of its PTA-nitrogen, and 10 per cent of its combined sugars. These averages based on 4 similar preparations are shown in the lower lines of Table I. In estimating the material balance between the sacs and their initial dialysates, it was found that all but 10 per cent of the original precipitable nitrogen had been recovered but that 30 per cent of the combined sugars had been lost from the system. At the same time, there appeared to be a compensatory gain in the smaller nitrogenous and reducing substances, indicating that larger molecules had been converted into smaller ones.

After "hydrostatic" dialysis against 30 to 35 L. of distilled water had been allowed to proceed for an additional 2 or 3 days, the losses through the cellophane had mounted; after a final exposure of the extracts to electrodialysis for 96 hours, only 22 to 31 per cent of the original combined sugars remained in the sacs of XVII No. 2, No. 6, No. 7, and No. 8 (Table II). The precipitable nitrogen dropped to between 13 and 21 per cent, the reducing substances to

TABLE I. DISTRIBUTION OF NITROGENOUS AND CARBOHYDRATE CONSTITUENTS OF WHOLE RAGWEED EXTRACT BETWEEN INITIAL DIFFUSATE AND CELLOPHANE SAC CONTENTS
(AFTER 2 TO 3 DAYS OF DIALYSIS AGAINST 1 OR 1½ VOLUMES OF DISTILLED WATER)

STARTING MATERIAL	RELATIVE VOLUME OF DIALYSANT	TOTAL RECOVERY OF MATERIAL FROM DIALYSATE AND SAC CONTENTS (EXPRESSED AS PER CENT)							
		COMBINED SUGARS (EXPRESSED AS GLUCOSE)		PTA-PRECIPITABLE NITROGEN		REDUCING SUGARS (EXPRESSED AS GLUCOSE)		NONPRECIPITABLE NITROGEN	
		DIALYSATE	SAC CONTENTS	DIALYSATE	SAC CONTENTS	DIALYSATE	SAC CONTENTS	DIALYSATE	SAC CONTENTS
Whole XVII No. 2	1	7	47	21	61	35	80	48	53
Whole XVII No. 7	1½	13	66	33	60	64	72	57	65
Whole XVII No. 8	1½	13		29		53		80	
Whole XVII No. 9	1½	5		29		53		85	
Average dialysate		9½		28		52		68	
Average sac contents			56		60		76		59
Average recovery from dialysate + sac contents		66		88		128		127	

between 7 and 9 per cent, and the nonprecipitable nitrogen was present only in traces (1 to 4 per cent). Although the missing materials were roughly accounted for in the accumulated dialysates, the figures for all but the initial preparations were considered doubtful due to the small amount of solids in the samples and the large factors by which the results had to be multiplied.*

TABLE II. PER CENT OF ORIGINAL EXTRACT REMAINING IN SAC CONTENTS AFTER ELECTRODIALYSIS OF RAGWEED EXTRACTS XVII

EXTRACT NUMBER	COMBINED SUGARS (GLUCOSE) (PER CENT)	PTA-N (PER CENT)	REDUCING SUGARS (GLUCOSE) (PER CENT)	NONPRECIPITABLE NITROGEN (PER CENT)
2	22	13	7	4
6	25	18	7	4
7	30	16	9	2
8	31	21	8	1
Range	22-31	13-21	7-9	1-4

Since, for reasons to be given later, the PTA-N and combined sugars (CS) would theoretically be better guides to potency than would the smaller molecular constituents (nonprecipitable nitrogen and reducing sugars), it will be of interest to know the relationships between the 2 in various fractions. The PN:CS ratio in whole extract XVII was about 1:5. More PTA-N than CS escaped into the initial dialysate, bringing its ratio to 1:1½. The ratio was overcorrected in the hydrostatic dialysate, being found at 1:7. At the conclusion of electrodialysis, the final product showed a marked reduction in PTA-N, while the CS had been lost at about the same rate as in the preceding, hydrostatic sample, the PN:CS ratio now being 1:18 in the electrodialysate.

The technique followed in preparing an initial dialysate appeared to influence its chemical make-up. Phosphate buffer, for example, withdrew as much nonprecipitable nitrogen as did distilled water but was less efficient in extracting PTA-nitrogen and particularly in bringing out carbohydrates, as shown by comparing first dialysate with the XVII dialysate in Table III. Secondary exposure of the sac contents used for first dialysate to barbital buffer at pH 7.9 yielded a diffusate with equal content of combined sugars but with only one-sixth as much precipitable nitrogen per cubic centimeter as its predecessor. The most striking differences were encountered when simple suction was used to procure filtrates for whole extract. Since no dilution was involved, naturally all constituents were present in higher concentration than when a dialysant was used. The ratio, however, between precipitable nitrogen and combined sugars was nearly 1:5, whereas in the initial dialysates of XVII, it did not exceed 1:2. About 40 per cent of each of these 2 components had apparently been pulled out of whole extract XIV without disturbing their original relationship. It will be recalled that only 10 per cent of the combined sugars initially escaped during conventional dialysis (Table I), whereas the loss of PTA-N was nearly 30 per cent. Whole extracts XIV and XVII, like their electrodialyzed sac contents, did not differ significantly, showing that the manner of preparation of the initial diffusates was responsible for the chemical differences rather than some variable in the pollens used.

*Detailed analytical results will be supplied on request.

TABLE III. CHEMICAL COMPOSITION OF INITIAL, SECONDARY, AND VACUUM-PREPARED DIFFUSATES OF LOW RAGWEED EXTRACT

PREPARATION	RELATIVE VOLUME OF DIALYSANT	DIALYSANT	DURATION (HOURS)	CHEMICAL COMPOSITION OF DIALYSATE			
				COMBINED SUGARS (MG. GLUCOSE/C.C.)	PRECIPITABLE NITROGEN (MG./C.C.)	REDUCING SUGARS (MG. GLUCOSE/C.C.)	NONPRECIPITABLE NITROGEN (MG./C.C.)
XVII No. 7, First dialysate	1½	Aqua destillata	87	0.5	0.24	2.1	0.25
First dialysate of 1/31/49	2	Phosphate buffer, pH 7.5	18	0.2	0.15	0.9	0.26
Second dialysate of 1/31/49	3.2	Barbital buffer, pH 7.97, ionic .1	46	0.2	0.025	0.3	0.04
Vacuum dialysate XIV	suction	none	17 days	3.6	0.77	4.5	0.58

Activity Tests.—As a preliminary to comparisons between sac contents and dialysates, experiments were set up to determine whether the chemical differences noted previously between vacuum dialysate XIV and first dialysate in phosphate buffer influenced their potencies. Among 22 patients concurrently tested with the 2 by conjunctival test, only 6 individuals showed any difference in the 2 threshold requirements. From 4 to 6 times more PTA-nitrogen was demanded of first dialysate by these exceptional cases. Since, as will be shown later, certain active substances were present in vacuum dialysate which were lacking in the first dialysate, the occasional difference noted in potency may have been the outcome of selective susceptibility to the extra antigens.

More extensive comparisons of potency were now drawn between one or other of the dialysates and whole extracts by means of serologic, conjunctival, and intracutaneous tests done on members of an experimental group of 125 ragweed-allergic patients. As shown in Table IV, Prausnitz-Küstner experiments on the pretreatment sera of Group A revealed that one-third of them could be made to give +++ responses to original extract if this allergen were injected in a strength of 40 units. First dialysate, on the other hand, had to

TABLE IV. SHOWING THAT LESS PTA-N IS REQUIRED FOR SKIN, EYE, AND PRAUSNITZ-KÜSTNER RESPONSES TO WHOLE RAGWEED EXTRACT OR ELECTRODIALYZED SAC CONTENTS THAN TO DIALYSATE

	PTA-N UNITS PER C.C.	CASES	SENSITIVITY CLASSES (PER CENT) +++	++	+	±	NEGATIVE
Serologic (Prausnitz-Küstner) Tests in Group A							
Whole XII	40	15	27	40	33		
First dialysate of 1/31/49	160	35	31	26	20		22
Sac contents XI	40	15	40	47	13		
Intracutaneous Tests in Group B							
First dialysate	160	57	32	14	17	17	20
Whole "standard," glycerinated	1	23	43	17	17	22	
Conjunctival Tests in Group C*							
First dialysate	160	31	6	26	19	23	26
Whole XIV, glycerinated	40	31	16	45	26	13	
Conjunctival Tests in Group B*							
Whole XIV, glycerinated	40	14	28	43	29		
Sac contents XIV, glycerinated	40	14	51	43	7		
Conjunctival Tests in Entire Membership*							
Vacuum filtrate XIV, glycerinated	160	125	31	26	16	18	8
Whole "standard," glycerinated	40	125	26	43	22	9	

*Basis of classification: strength of solution giving rise to threshold reaction in eye.

	Class +++	Class ++	Class +	Class ±	Negative
Whole:	40 U or less	80 to 160	320 to 640	1280 to 10,240	
Dialysates:	160 U or less	320 to 640	1280 to 2560	5120 to 20,480	40,480

be employed in 160 unit strength to provide a corresponding number of marked reactions. The adoption of these 2 strengths as the standard test agents provided positive results with the mother solution in all instances, but negative reactions in 22 per cent of trials with dialysate. When sac contents were used in 40 unit concentration, a somewhat higher proportion of sera gave +++ reactions than with whole extract, indicating that the PTA-nitrogen left in the sac after electrodialysis had greater potency than that in the original extract.

Fifty-seven individuals were examined by direct intracutaneous tests. As in the case of the serologic studies, 160 unit dialysate served to classify patients, since it provided 32 per cent of +++ responses. There were 20 per cent of negative reactions. However, when 1 unit strength of our standard glycerinated whole extract was chosen as a basis for segregation, more cases fell into the +++ category than with 160 unit dialysate, and there were no negative results.

The data accumulated during conjunctival tests of the threshold variety on 31 individuals were handled somewhat differently. When the patients were listed in the order of their susceptibility, it was found that one-third of them reacted to glycerinated whole extract XIV in a strength of 40 units or less, whereas the test with first dialysate had to be carried to the 160 unit preparation before one-third of the group showed the standard reaction. The basis of distribution for the rest of the group, who were less highly allergic, is stipulated in Table IV. It also shows that no responses were encountered in 26 per cent of the group when dialysate was employed, whereas whole extract was active in all subjects without exception. Fourteen subjects evaluated with sac contents proved more reactive to this fraction than to the mother material in equal strength.

The entire membership of 125 was included in the final comparisons between standard whole extract and vacuum filtrate, both of which had been stabilized by glycerination. Using the same system of classification as that just described for conjunctival tests, it was found that at least 4 times more PTA-nitrogen usually had to be allowed for dialysate than for whole extract to elicit a comparable response. Whereas about one-third of the patients were classified as having marked allergy for dialysate, nearly 10 per cent of the members proved to be insusceptible to this fraction; all were affected by whole extract of 10,240 unit strength or less. Patients showing no conjunctival erythema until 40,960 unit vacuum filtrate had been instilled were considered unresponsive. Table IV shows that a lower incidence of negative reactions was obtained for vacuum filtrate than for conventional dialysate, a difference which might be referable to the more complex chemical and antigenic structure of the former, and to its stabilization with glycerine.

These relationships between the threshold doses of whole extract and of vacuum dialysate have been presented in the form of frequency curves (Fig. 1) and cumulative frequency curves (Fig. 2). Both show that more nitrogen is in general required of dialysate than of the mother solution, the distribution curves for the fraction being shifted to the right. No threshold response was encountered, for example, to a solution containing less than 40 units of dialysate per c.c., whereas 10 unit material was effective in a small proportion of the tests with

whole extract. At the other extreme, it was found that only 2 patients required antigen as strong as 10,240 units for their responses to whole extract, whereas 25 members needed from 10,240 to 40,960 for the comparable response to dialysate.

It will be seen in Fig. 2 that 90 per cent of the 125 patients responded to whole extract by the time 640 unit dilution had been instilled, whereas 20,480 unit dialysate had to be reached before 90 per cent of them reacted to this derivative. Since the end points in the conjunctival test can be reproduced within 1 tube and often within half a dilution step, this difference of 5 dilution tubes has definite statistical significance.

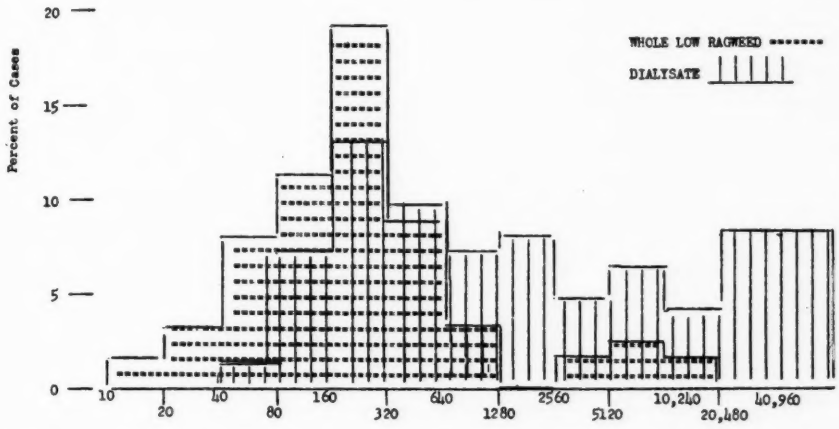

Fig. 1.—Threshold of conjunctival reaction to dialysate and to whole low ragweed extract in 125 patients prior to booster therapy.

Although a change in activity has obviously been brought about by fractionation, specificity differences must also be involved. The basis for this claim is that the over-all shapes of the curves for the two test solutions are different. It is therefore not tenable to assume that they differ merely in the potency of their PTA-N, that inert nitrogen from the mother solution has diluted the active nitrogen of the dialysate. The reason for the altered shape of the diffusate curve became evident when the individual threshold doses determined for the 2 materials were examined for successive members of the group. For 56 patients with marked susceptibility to filtrates, the dialysate: whole extract ratios ranged from $\frac{1}{8}$ to 40, with a median value of 3. At the other extreme were 10 patients who showed little or no response when tested with dialysate 40,960. Their dialysate: whole extract ratios ranged all the way from 4 to 500, with a median value of 85. Meanwhile the absolute values for whole extract did not exceed 320 among the first group, but were never less than 1,280 for the latter group. Thus, there was a general trend for the susceptibility to increase for 1 solution as it did for the other, but the dialysate: whole extract relationship varied widely with the individual.

The difference in shape of the 2 curves would presumably have been more pronounced had conventional dialysate rather than the more complex vacuum preparation been used for these comparisons.

A single antigenic mixture in the 2 solutions could not give rise to these wide variations. If the dialysate antigens were identical with those of whole extract but had been diluted with inert nitrogenous material which escaped during dialysis, then the shapes of the 2 distribution curves should be alike, and the dialysate data should have been merely shifted to the right by its lower potency. If, on the other hand, individual specificities were involved in the dialysate and in the sac contents, the observed difference in shape between mother extract and fraction would be expected.

Fig. 2 makes it clear that the median requirement was 160 for whole extract, 640 for dialysate, whereas the most probable requirement for either solution was 160 unit strength (Fig. 1).

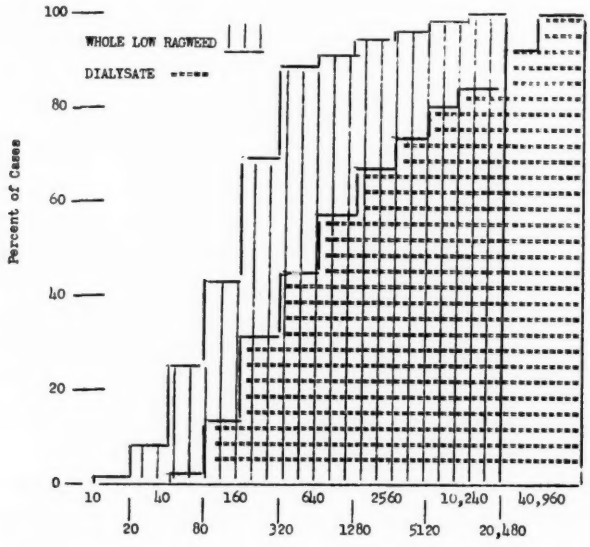

Fig. 2.—Threshold of conjunctival reaction to dialysate and to whole ragweed extract in 125 patients prior to booster therapy (cumulative).

Serologic and conjunctival comparisons for potency were carried out with the yields of conventional dialysis at its several stages. The initial dialysate of XVII possessed about twice the potency per PTA-N unit as the hydrostatic sample, and from 3 to 6 times the activity of the vacuum dialysate of XIV when both were examined in several Prausnitz-Küstner experiments as well as in 9 sets of eye tests. It was also stronger than the electrodialysate, from 1½ to 32 times less PTA-N being required of it for conjunctival responses in 10 patients, the median value being 4. Its potency in Prausnitz-Küstner studies appeared about 25 times greater than that of the final dialysate.

A puzzling situation arose when such comparisons were made between the first and second dialysates of the Extract of Jan. 31, 1949. Volume for volume, the activity of the secondary yield (in barbital buffer) was distinctly greater (tenfold) than its predecessor in phosphate buffer, in spite of the fact that it contained only one-sixth as much PTA-N. Furthermore, during neutralization

experiments it appeared that 120 units of second dialysate sufficed to exhaust the sensitizing power of a ragweed-sensitive serum (RO), whereas 1,280 units were required of first dialysate. No explanation was found for this reversal of the trend noted previously with the distilled water dialysates to become less potent per PTA-N unit as dialysis continued. It might be assumed that the 2 buffers played some selective role.

Heating of initial dialysates of XVII in a water bath at 60° C. and 100° C. had no observable effect on their potency, as judged by the minimal dose required for Prausnitz-Küstner responses. Electrodialyzed sac contents XVII No. 3, on the other hand, showed a loss of perhaps one-half its activity following exposure to 60° C. and further damage after 100° C.

Freeze-drying apparently did not reduce the potency either. The allergenic activity of the initial 1:2 dialysate of XVII No. 2 was as great after being lyophilized 1 or 4 times as it was in the original state. The same situation obtained for the initial and the hydrostatic samples of XVII No. 7 when the once-dried samples were compared with the 4 times lyophilized samples. Furthermore, no impressive differences in potency were noted between Prausnitz-Küstner reactions of 35 sera to fresh first dialysate (Table IV) and the responses of several sera to the lyophilized samples of XVII dialysates at a later date.

In addition to the foregoing evidence for the capacity of dialysates to combine with preformed sensitizing antibodies in the serum, skin, and eyes of certain pollen-allergic patients, confirmation was given by 2 subjects who developed prompt systemic reactions following slight overdosage with therapeutic injections of diffusate.

Cross Tests for Immunologic Specificity (First and Second Dialysates).— In spite of their chemical and potency differences, neither the first and second dialysates nor the initial, hydrostatic, and electrodialysates of XVII could be differentiated from each other in cross tests. Table V shows that a site of normal skin, previously injected with a mixture of serum and excess dialysate, reacted negatively to test with either the homologous or the heterologous dialysate in high concentrations. The same relationships obtained for the successive diffusates of XVII. By contrast, any such site would readily respond to retest with dilute sac contents or whole extract, indicating the satisfactory state of the area and showing that the dialysates were simplified fractions. Sites which had been injected with serum-saline mixtures responded to very small amounts of precipitable nitrogen when second dialysate, sac contents, or whole extract were injected, whereas many more gamma of precipitable nitrogen per cubic centimeter had to be used for equivalent results with first dialysate. None of the testing solutions was nonspecifically irritating, as control tests in normal areas revealed.

Cross tests between heated and unheated samples of XVII dialysates showed that the heat had failed to reduce their antigenic complexity, the diffusates heated to 100° C. exhausting a sensitized site completely toward retest with either the 60° C. heated specimen or the unheated solution. Similar experiments with heated sac contents XVII suggested that certain of the nondiffusible

antigens were more effected by heating than others. A similar situation was encountered in the case of XVII dialysates following their exposure to freeze-drying, especially when it was repeated as often as 4 times. This destruction of some of the antigenic components was not sufficiently widespread to be reflected in the less delicate potency tests referred to previously.

TABLE V. SEROLOGIC CROSS TESTS SHOWING ANTIGENIC SIMILARITY OF FIRST AND SECOND DIALYSATES AND THEIR SIMPLIFICATION OVER SAC CONTENTS AND WHOLE EXTRACT (TEST SUBJECT SW; REACTION TO 24 HOUR TEST WITH ANTIGEN)

Antigen mixed with RO Serum	1st Dialysate (mg Pta-N/cc)	2d Dialysate (mg Pta-N/cc)	Sac Contents XI (mg Pta-N/cc)	Whole Extract XII (mg Pta-N/cc)
1st Dialysate	0.15 / 0	0.02 / 0	0.0004 / 1½	0.00015 / 3-
2nd Dialysate	0	0	2	3-
Serum-saline control	.015= 2½ .150= 3	.0001= 2½ .0030= 3	.0001= 3 .	.00015= 3
Normal skin control	.150= ±	.0300= 0	.0004= 0	.00150= 0

Sac contents, dialysate and whole extract: Table VI covers the cross test results of first dialysate, electrodialyzed sac contents XIV, and whole extract XII, all of which were prepared within a few weeks' time. As would be expected, a serum-antigen mixture containing an excess of the starting material failed to respond next day to test with either of the derivatives, sac contents and a diffusate of either the conventional or vacuum type. On the other hand, sites neutral to sac contents still responded partially to whole extract or either of the filtrates. In the reverse test, a vacuum filtrate site was one-half reactive to whole or sac contents but not to first dialysate. The vacuum diffusate therefore must have lacked activities present in whole extract and in sac contents, but contained all the antigens which made up the initial dialysate. When the 2 dialysates were used in the opposite order, a half-maximal wheal developed after vacuum filtrate was introduced. This indicated that it was less simple than first dialysate, confirmation for which was found in the nearly maximal results obtained with whole extract and with sac contents in these first dialysate sites,

whereas only partial responses had been detected in the vacuum dialysate sites. To all appearances, suction is a less desirable means of preparing diffusates than is conventional dialysis.

Three sera bearing no sensitizing power for dialysates were mixed with large amounts of first dialysate and were subsequently found to be maximally reactive to cross test with sac contents and with whole extract, showing again that the nondiffusible specificities differed importantly from the diffusible.

TABLE VI. DIFFERENCE IN SPECIFICITY OF DIALYSATE, SAC CONTENTS, AND UNFRACTIONATED EXTRACT OF LOW RAGWEED POLLEN (TEST SUBJECT HEL; RESPONSE TO 24 HOUR TEST)

Antigen mixed with BAU Serum in vitro	Whole Extract (mg Pta-N/cc)	Sac Contents (mg Pta-N/cc)	Vac. Filtrate (mg Pta-N/cc)	1st Dialysate (mg Pta-N/cc)
Whole Extract XII	0	0	0	0
Sac Contents XIV	1/2—	0	1/2—	1/2—
Vacuum Filtrate XIV	1/2	1/2—	0	0
1st Dialysate	max—	max—	1/2	0
BAU Serum-salt	.00014= 3½	.00008= 2 .0008 = 2½	.0008= 2 .0016= 2½	.01280= 0 .15400= 2½
Normal skin	.10000= 0	.01800= 0	.01280=0	.01280= 0 .15400= ±

After obtaining the same cross results among whole extract, sac contents, and filtrates with 6 different dialysate-allergic sera, it was concluded that common as well as individual activities were possessed by each derivative. In other words, although diffusible had been separated from nondiffusible antigens during dialysis, the process had not been carried quite to completion, residual specificities of the small molecular type still being detectable in the sac contents after electrodialysis. At the same time, certain large molecular substances remained exclusively behind in the sac contents. Sera neutralized with either of the simplified derivatives could still respond to the starting extract by virtue of

its more complex makeup; but the reverse test was always negative. Thus, dialysis effected separation into 2 composite fractions with some overlapping between the 2.

When sac contents and filtrate were both added to the mixture, there was no response to later tests with whole extract, indicating that original specificities had been retained throughout fractionation.

Immunizing Power of Dialyzable Components.—Eight patients with ++ or +++ dialysate-allergy were given vacuum filtrate therapeutically in a large single dose (1,600 to 3,000 PTA units, median 2,000) which was mixed with the individual's own serum in 6 instances and with saline solution in 2 control cases. Each mixture was incorporated into a water-in-oil emulsion before being injected into a subcutaneous depot. One patient in each group developed a mild, classical, systemic reaction of overdosage. One subject in each category received no therapy in addition to the depot, whereas the rest of the patients were also given a later injection with a water-in-oil preparation of whole extract. Judged by periodic conjunctival tests, the antibody response to dialysate during the next several months were neither negative nor consistently positive. The maximal increase in the threshold requirement of vacuum dialysate noted for the 2 controls given filtrate-saline depots amounted to 2 and 6, respectively. Three subjects given serum-filtrate mixture showed ratios no higher than 2, which we considered of no significance; 1 showed a change of threefold, and another of 12 fold. One patient appeared to have increased his sensitivity, for his threshold dose dropped threefold. The ratios for the entire group ranged from ⅓ to 12, median 2½. The 6 subjects meanwhile were responding rather better to their depots of whole extract or sac contents, maximal ratios for the whole extract test solution ranging from 2 to 32, with a median value of 4. Thus, by inference, blocking antibodies were formed somewhat more efficiently for the mother solution than for the fraction following the use of a large, complementary dose of vacuum dialysate in addition to whole extract or sac contents.

These ratios were compared with others concurrently set up for 39 ++ and +++ dialysate-allergic patients who took only whole extract in their single depot injections. Vacuum filtrate was used in conjunctival tests to determine their threshold doses during the subsequent few months and revealed that 61 per cent of the group remained unchanged, with ratios of 1 or 2, whereas for 29 per cent these ratios ranged between 3 and 12, and for 10 per cent reached 16 to 64. Thus, although the experimental group of 8 was too small to permit final conclusions, it appeared that the serum-dialysate mixture had been somewhat less efficient than whole extract in setting up immune responses toward the diffusible antigens of ragweed pollen.

It was our impression, also, that the patients tolerated larger doses of filtrate when it was mixed with reaginic serum than with saline solution. We had predetermined for each serum-dialysate mixture approximately how much "free" dialysate was available per cubic centimeter by evoking comparable conjunctival responses with it and with plain dialysate in the patient who was to receive the mixture. At least 80 per cent of the activity appeared to have

been bound by the 6 sera. The author has shown that pollen reagin (admixed with extract) can prevent the allergen from eliciting responses in the allergic eye,[12] presumably by binding the antigen in vitro.

It was incidentally observed, among a larger group of 84 whole extract-treated patients with various reactivities to filtrate, that 8 developed an increase in susceptibility for diffusate by eye test, the degree ranging from 3 to 30 fold. The ratios of increased resistance for whole, meantime, ranged from 1 to 170 in this large group, with a median value of 8, showing that unfractionated extract was a more effective immunizing agent than dialyzable antigens whether or not they had been admixed with reaginic serum (median ratio, 2½).

The clinical immunity of the filtrate-inoculated patients did not appear changed significantly from earlier years when depots of whole extract had been administered in equal or smaller doses. If these preliminary findings with injected serum-filtrate mixtures and with filtrate-containing whole extract can be confirmed, some explanation must be sought for the failure of diffusible antigens admixed with reaginic serum to enhance clinical immunity, in the face of their demonstrated capacity to develop conjunctival resistance in a sizable minority of patients given the mother solution without serum. It is, of course, possible that our reaginic serum covered up the active groupings of these haptens and thereby discouraged antibody responses to the serum-filtrate mixtures. One might anticipate that such a mechanism would, however, also have operated in vivo during treatments with whole extract, thereby preventing the observed increases in conjunctival tolerance. Further studies are indicated.

Electrophoretic Patterns.—In Fig. 3, comparison can be made between the electrophoretic patterns of undialyzed whole extract and of a concentrated sample of first dialysate. The dialysate is less complex in constitution, containing only 2 well-defined peaks, whereas whole extract contains a broadly distributed spectrum of mobile pigmented components in addition to the large immobile peak.

The marked lack of symmetry in the ascending and descending patterns, as well as in the pigmented peak, may be in part due to imperfect equilibration of the fraction with the buffer. It will be recalled that dialysis against the Tiselius buffer was curtailed in duration and modified by the use of 7 thicknesses of cellophane in order to discourage loss of the readily diffusible material while the salt concentrations were becoming equalized. Because the resistance of the buffer was found to differ from that of the sample, reliable absolute value of mobilities could not be determined. This may also have given a false impression of the area of the immobile component, since it is superimposed on the anomaly and the latter may be abnormally large due to imperfect equilibration. In order to decide the question of heterogeneity of either peak, reversible boundary spreading experiments (Alberty[2]) would have to be done.

Although the use of averages for estimating the concentrations of components is somewhat doubtful, for reasons just given, when such estimates are made, 31 per cent of the constituents of whole extract appear to be mobile and 69 per cent immobile at pH 7.5, whereas the corresponding areas of dialysate are 5 per cent and 95 per cent, respectively. The patterns obtained with sac

contents were so low, due to loss of substance during the prolonged dialysis of fractionation, that the photographs will not be presented. Direct visualization of the magnified peaks during the 2½-hour run at pH 7.5 showed a major, very slowly moving, colorless component and a mobile, colored constituent which was heterogeneous. Although it appeared to be less complex than the pattern for undialyzed whole extract, definite conclusions must await the analysis of a concentrated sample of both preparations. However, it was estimated that dialysis had effected a reduction in the immobile components to about 47 per cent of the total area while a proportionate increase had taken place in the mobile constituents until they comprised 53 per cent of the area.

These preliminary analyses show qualitatively that the shift of material through cellophane into the dialysate had been selective, involving largely those substances which were electrophoretically immobile at pH 7.5.

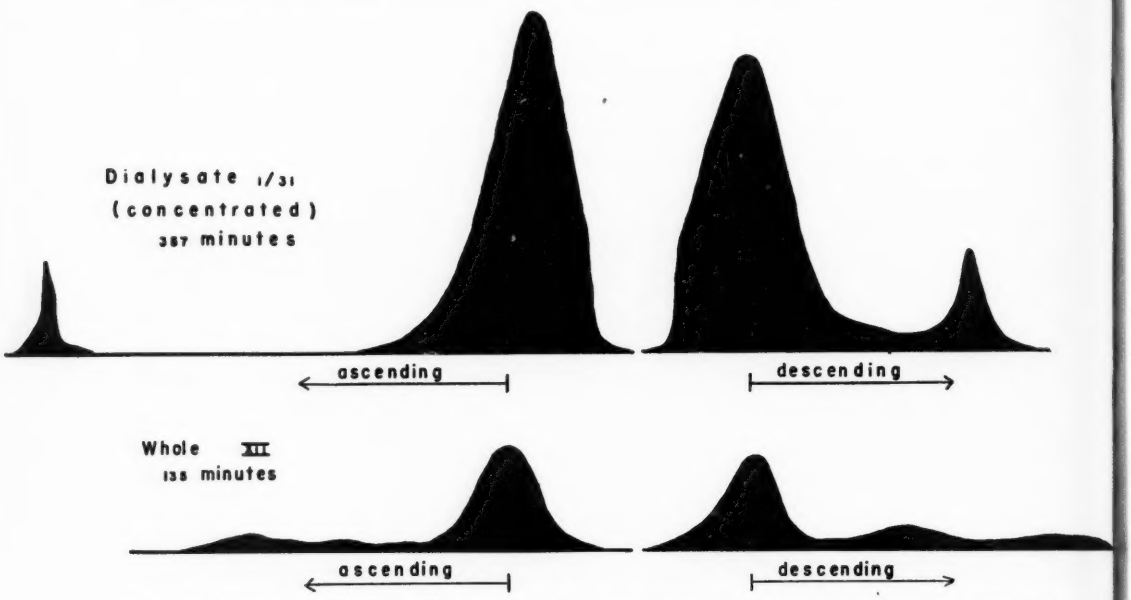

Fig. 3.—Tiselius electrophoretic patterns of low ragweed extracts (phosphate buffer of ionic strength 0.10, pH 7.50).

The sample of dialysate used for the Tiselius run contained 50.5 mg. of solids per milliliter (after excluding the weight of its buffer salts), of which 8 per cent was reducing sugars, 2.4 per cent was nonprecipitable nitrogen, 1.7 per cent was combined sugars expressed as glucose, and 1.3 per cent was PTA-N. Since the initial dialysates contained a much higher proportion of PTA-N to combined sugars than did the mother material (Experiment XVII), their PN:CS ratios being 1:1½ and 1:5, respectively, it might be deduced that the outward shift of immobile material into the dialysate was concerned more importantly with precipitable nitrogen than with the combined sugars. Polypeptids and smaller forms of nitrogen down to amino acids would be brought down by phosphotungstic acid and might be assumed to be the types involved. It is, of course, not possible to exclude the reducing sugars and nonprecipitable nitrogen

as factors contributing to the increase in the immobile components of the dialysate, since these substances diffused out quickly and freely through the cellophane and could quite possibly have been visualized by the electrophoretic technique.

Cross tests of electrophoretic fractions: Samples of the fastest- and slowest-moving materials were withdrawn from the Tiselius apparatus at the conclusion of the runs on first dialysate and sac contents so that their relative specificities could be appraised by means of serologic cross tests. Sites which had been injected with a serum-antigen mixture containing an excess of the fastest-moving, dialyzable fraction (pigmented) still responded to cross test with its slowest fraction (uncolored), and vice versa. These reactions were read as 1½ plus (+±) and probably were about half-maximal, although sufficient material was not available to establish this definitely in serum-saline sites. Either site reacted at least half-maximally when tested with the fastest component of sac contents, showing that mobile activities had been retained by the cellophane which differed in specificity from both the immobile and mobile substances that diffused out. In the converse experiment, a site exhausted with the fast material from sac contents responded ++ to retest with the fast fraction of first dialysate. Since these 2 way tests with nonirritating solutions provided mutually positive results, it could be concluded that each electrophoretic fraction possessed at least one specificity not present in the other. The supply was insufficient to determine whether they also contained mutual antigens, but this situation had been encountered in cross-tests between the unfractionated sac contents and dialysate (Table VI).

From these limited immunologic comparisons, evidence is at hand that low ragweed pollen contains at least 3 different specificities separable by cellophane and electrophoresis: 2 in the dialysate which differ from a third, mobile component of the nondiffusible portion of the extract.

Chromatography.—Cross tests among the eluates taken from the several crucible holders revealed that 3, and probably 4, different antigens were present in the initial dialysate of XVII. When, for example, normal skin was injected with a mixture of standard sensitizing serum and the pale yellow filtrate which had emerged from the lowermost crucible holder, it proved unresponsive next day when tested with this filtrate in undiluted form (PTA-N 0.17 and CS 0.6 mg. per ml.). An identical site reacted half maximally, however, to test with a yellow eluate taken from the upper portion of the second holder, fraction No. 2-a, which contained only 0.01 mg. PTA-N per ml. In the reverse experiment, No. 2-a inactivated sites toward cross test with undiluted filtrate. Thus, No. 2-a must have contained all the allergens of the filtrate, plus at least one additional activity. In spite of its complexity, the eluate was found to be simpler than another pigmented fraction eluted from the lower half of the top holder, fraction No. 1-b, which had a PTA-N value of only 0.02 and a CS content of around 0.2 mg. per ml. and which contained all activities present in No. 2-a and the filtrate, as well as a distinctive antigen. This showed that no fewer than 3 entities had been originally introduced into the adsorption column. Since

each of the foregoing derivatives failed to inactivate its sites for the unfractionated dialysate, one could deduce that at least 4 activities were involved in the starting material, initial dialysate XVII. Serum-saline control tests indicated that the faintly tinted filtrate was less potent than an equal volume of the definitely colored eluates, in spite of its higher content of both PTA-nitrogen and CS.

SUMMARY AND DISCUSSION

Phosphate buffer extracts of low ragweed pollen have been divided into 2 portions by means of suction or dialysis through commercial cellophane membrane. The diffusates differ from the sac contents in their carbohydrate: nitrogen ratios, in antigenic potency as well as specificity, and in electrophoretic constitution. Although both derivatives were simplified over the starting material, whole extract, neither was single as judged by electrophoretic, immunologic, and chromatographic analysis.

Confirmation was found for the claims of others[3, 4, 7, 8, 11, 13, 16-18, 20] that pollen materials and activity diffuse out through cellophane. The extent of these losses has been approximated for the first time by the expedient of saving all yields of prolonged dialysis for a number of identical experiments. During initial dialysis against 1 or 1½ volumes of distilled water, the several phosphate buffer extracts of XVII lost about two-fifths of their nonprecipitable nitrogen, one-fourth their reducing sugars, two-fifths of their phosphotungstic acid-precipitable nitrogen (PTA-N), and 45 per cent of their combined sugars (CS). Meantime, some 30 per cent of the combined sugars and 10 per cent of the PTA-N had been converted to reducing sugars and nonprecipitable nitrogen, respectively. Although the extent of these gains in smaller molecular material at the expense of larger substances was not great, the shifts were suggestive of a possible breakdown of enzymatic or other nature taking place in the extract at 4° C. and under sterile conditions.

By the conclusion of 5 or 6 days of additional exposure of the sac contents to hydrostatic dialysis and to electrodialysis, the losses into the diffusate had mounted. Indeed, the residual extract possessed only 25 per cent of its original combined sugars, 15 per cent of its PTA-N, 8 per cent of its reducing sugars, and a trace of its nonprecipitable nitrogen. Meanwhile, an attempt to establish a crude material balance revealed that approximately one-fourth of the original PTA-N had been lost from the system while a compensatory gain of over 10 per cent had taken place in nonprecipitable nitrogen. An excess of free reducing sugars was also recovered.

The potency per PTA-N unit was reduced in the initial dialysate as compared to whole extract, all but a few of the 125 ragweed-allergic subjects tested by the conjunctival method requiring decidedly more nitrogen of the diffusate than of the whole extract. In patients showing minimal susceptibility to dialysate, the requirement was 500 times that of the unfractionated extract. These findings show not only that the diffusible nitrogen possesses a generally lower potency but also that the susceptibility for sac contents and for diffusate varies independently. Hence, different specificities must be involved. As dialysis

proceeded, the yields showed an even greater reduction in their potency as gauged by conjunctival and Prausnitz-Küstner tests, the hydrostatic product possessing about one-half the activity of its predecessor, the initial dialysate, and the electrodialysate having only a fraction of their potency per PTA-N unit.

An effort was made to correlate these shifts in potency with shifts in the 4 chemical constituents of the fractions. Since over 90 per cent of the reducing sugars and nonprecipitable nitrogen were removed from the extract during dialysis and could be roughly accounted for in the accumulated diffusates, and since the dialysates proved in general far less potent per unit of carbohydrate or of nitrogen than did the sac contents, one could conclude that these highly diffusible, small substances played no significant role in activity. This leaves the better retained PTA-N and combined sugars for consideration. The PN:CS ratio changed as follows: from 1:5 in whole extract to 1:1½ in the initial dialysates, to 1:7 in hydrostatic dialysates, and to 1:18 in the final electrodialysates. Although the concentration of PTA-N and the potency per nitrogen unit progressively fell in successive diffusates, rendering this assay method an unsatisfactory index to activity, the carbohydrate content was even less reliable inasmuch as the PN:CS ratio rose as the concentration and potency of the PTA-N steadily decreased. If the precipitable nitrogen was a determinant of activity, it must have been accompanied by considerable and increasing proportions of inert PTA-N as it found its way out of the sac. Spain and Newell[17] found that 20 to 30 per cent of the PTA-N of ragweed extract diffused through heavy cellophane and was inactive. Meantime, our sac contents was enhancing somewhat its activity per PTA-N unit and its PN:CS ratio, the latter reaching 1:8 in the final, electrodialyzed sac contents. Either assay method therefore could have served as a reasonably satisfactory means of reference to activity in successive sac contents. For the whole extract, no single chemical procedure was ideal due to the selective susceptibility of patients to dialysate, but the PTA-N content appears to follow activity more closely on the average than does CS.

A few speculations are permissible regarding the relationship of these chemical and activity characteristics to the electrophoretic results. The striking difference between the whole extract and its derivatives was a change in the relative proportions of the mobile and the immobile constituents, the latter increasing from 69 per cent in whole extract to 95 per cent of the total area in dialysate and being reduced by a like amount in the sac contents. Inasmuch as the immobile component of the diffusate was found to be serologically active, and it has been reasoned that the PTA-N is the most likely chemical category to account for the allergenicity of diffusates, it might be inferred that the PTA-N contributed activity and area to the immobile component of the dialysate. By way of contrast, the slowest-moving unpigmented material (SU) remaining behind in the final electrodialyzed extract at pH 7.5 appears to possess little activity, as will be shown in a later publication. Furthermore, this retained SU material contains considerable carbohydrate and some PTA-N, if the limited assays done to date can be reproduced. Since the filtrate delivered from the chromatographic colums still possessed considerable CS and PTA-N, some

color, and only moderate potency per PTA-nitrogen unit, one might conclude tentatively that at least part of the PTA-nitrogen in our conventional diffusates was relatively inert. One might speculate that the small amount of activity possessed by this filtrate from the adsorption column might depend on its trace of pigment. The latter differed from other pigmented allergens in the dialysate by its inability to be absorbed on alumina at pH 5.

It would be profitable to establish the relationship of our findings to those of others in the literature. Abramson, Moore and Gettner, like Rockwell, attribute the allergenicity of ragweed pollen chiefly to its diffusible component of 5,000 molecular weight. Nevertheless, only 9 of their 15 patients reacted when this SU fraction was sprayed into their nostrils. This places it in the category of Stull's dialyzable fraction 2 and of our dialysates, which elicited responses in only a portion of the ragweed-allergic population. In view of the fact that undialyzed phosphate buffer extract had been employed for Abramson's electrophoretic separations, one is perplexed that no mention is made of any high molecular weight constituents. Both Stull and the writer have reported nondiffusible allergens in this pollen which are more regularly and more potently active than are the diffusible materials. Newell also found activity and an SU component in his electrophoretic analyses of whole extract which had been dialyzed for 20 hours, and in all 3 of Stull's fractions, only 1 of which (Fraction 2) is diffusible.

Rockwell further held that maximal protection from hay fever has been procured by patients given his 4,500 molecular weight fraction as an immunizing agent. This stands in contrast to the findings of Stull and associates with fraction 2 and of the writer who employed both ultrafiltrates[11] and suction-diffusates therapeutically. Since diffusible antigens are only selectively active, have lower potency, and are far less efficient in immunization, one would assume that they are of less over-all importance to patients with hay fever than are nondiffusible constituents in the pollen.

The incompatible results of Abramson and associates and of Newell with reference to the diffusibility of the slow, unpigmented components have been reconciled by our finding of a similar major electrophoretic constituent in both the dialysate and the electrodialyzed sac contents. Although the 2 could readily be confused if one examined but 1 of them in a given electrophoretic experiment, they can be distinguished when both halves of the extract are analyzed concurrently. The diffusible type remained stationary throughout a 6 hour period of observation at pH 7.5, whereas the nondiffusible SU moved very slowly during a shorter run. The former was active, the latter questionably so. Since the 20 hour dialysis of Newell[13] failed to rid the extract of its SU and our prolonged electrodialysis removed only a portion of this major component from the sac, Abramson's undialyzed fraction must have contained both the diffusible and the nondiffusible varieties of colorless material.

Although many attempts have been made during the past half century to isolate an allergen from pollen, no one prior to the present report has come

closer to this goal than Abramson's group. Their electrophoretic separation would have been an outstanding achievement if it had been associated with proof as to the fate of the nondiffusible components of the pollen as well as immunologic evidence of purity.

The significance and implications of our own findings would appear to be as follows: Although pollen is a highly complex mixture of chemical, electrophoretic, and immunologic entities, an almost complete separation of diffusible from nondiffusible activities has been effected by means of dialysis through Cellophane membrane. The majority of the material to escape was electrophoretically immobile at pH 7.4. Attempts to refractionate the diffusate by electrophoretic and chromatographic means yielded at least 3 different excitants. The major electrophoretic component was colorless and contained at least 1 antigen which was serologically different from its colorless, major, very slowly-moving counterpart left inside the sac. Since patients show a marked degree of difference in their relative susceptibility to the diffusate and to the sac contents, it is to be expected that they may also show selectivity in their responsiveness to the various constituents of either of these complex fractions. In order to understand the exact relationships between clinical allergy for pollens and the presence of sensitizing antibodies in the patient, it will therefore be indicated in the future to isolate each of the numerous excitants in pollen and to utilize these to determine the titer of each related reagin. This means that the antigen must appear homogeneous by several criteria: namely, its behavior in the ultracentrifuge, in diffusion studies, in the electrophoretic field, as well as in its chemical composition and its immunologic specificity. One or more of these criteria may then be found to serve as a truly reliable means of reference, to replace the present unsatisfactory chemical and "weight-by-volume" indices of potency.

The next step toward the isolation of excitants from pollen will be the refractionation of our 2 composite fractions, diffusate and sac contents. Gentle physical methods which will not risk denaturation would seem preferable to older, chemical methods. This laboratory will soon publish its pioneering experiments with electrophoresis-convection in the case of the nondiffusible portion of the extract. It will be of particular interest to re-examine in greater detail the chemistry, specificity, and potency of the 2 major components (SU) of the sac contents and diffusate, so as to establish clearly their similarities and differences. Some means should be sought for ridding the fractions of this ubiquitous major unpigmented material, so that other antigens may be more easily obtained in pure form. The several pigments inside and outside the dialyzing sac must also be separated from each other in quantities sufficient for characterization.

When one or more isolated antigens have been procured, it will be possible to explore the relationships of the spectrum of sensitizing antibodies to clinical allergy and to evaluate the connection between the related thermostable, or blocking, antibodies and clinical resistance. Eventually, it should be possible to contribute further evidence for the theory that allergic man is capable of

forming 2 competitive types of antibody for the same single antigen, one being concerned with hypersensitiveness whereas the other is capable of increasing clinical tolerance.

It is urgent that all fractionations and characterizations be carried out under rigidly controlled conditions, since the outcome may be importantly influenced by such factors as aging, heat, freeze-drying, and contamination by molds or bacteria. Furthermore, the nature of the diffusate varies with the use of suction, certain buffer salts, and especially with the susceptibility of the human subject selected for the potency tests.

CONCLUSIONS

1. An appreciable proportion of the large losses of nitrogen and carbohydrate from low ragweed extract during extensive, conventional dialysis through cellophane might be due to the breakdown of larger into smaller filtrable forms. The extent of this conversion has been approximated for the first time by establishment of a material balance. It amounts to 10 per cent for the nitrogenous materials and to about 30 per cent for the carbohydrates. It occurs at 4° C. and under aseptic conditions.

2. The total losses from the sac during dialysis and electrodialysis amounted to approximately 98 per cent of the nonprecipitable nitrogen, 92 per cent of the reducing sugars, 85 per cent of the phosphotungstic acid-precipitable nitrogen, and 75 per cent of the combined sugars.

3. Whereas all ragweed-allergic patients reacted to the nondiffusible constituents, at least 20 per cent of them were unaffected by diffusates in extensive tests of the skin, eye, and serum.

4. Diffusates differed from nondiffusible constituents in potency per PTA-N unit as gauged by intracutaneous, conjunctival, and serologic tests. Their specificities were also different as shown by serologic cross tests, as well as by concurrent eye tests with whole extract and diffusate in 125 allergic subjects whose susceptibilities to the 2 fractions varied independently.

5. Electrophoretically, the initial dialysate differed from whole extract in its greater proportion of immobile component at pH 7.4. The sac contents showed a corresponding reduction of this area.

6. Successive diffusates showed a loss of potency per PTA-nitrogen unit as dialysis proceeded and a progressive increase in the precipitable nitrogen: combined sugar ratio. The sac contents showed a slight increase both in potency per PTA-N unit and in PN:CS ratio.

7. Cellophane dialysates, although simplified over whole extract, contained 3 and possibly 4 chromatographic antigens, at least 2 electrophoretic entities. Not less than 1 additional antigen is possessed by sac contents.

8. Confirmation was found for the claim[1] that pigmented, mobile material as well as unpigmented, slow-moving substances of low molecular weight in low ragweed pollen have allergenic activity and that both nitrogenous and carbohydrate materials diffuse out through cellophane.

9. Diffusates were not only less potently and less frequently allergenic than whole extract and sac contents, but were less efficient as immunizing agents in

the production of thermostable antibodies and of clinical resistance. This confirms the opinion of Stull and his associates but is in disagreement with that of Abramson and Rockwell, who hold that molecules of about 5,000 molecular weight are the chief allergens of ragweed pollen.

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SMALL INTESTINE ROENTGEN STUDIES IN FOOD ALLERGY*†

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THE study of gastrointestinal allergy has been handicapped by the lack of reliable objective diagnostic aids. Skin tests have been unreliable, elimination diets difficult and cumbersome to apply and often inconclusive. Special testing techniques involving the study of white blood cell counts before and after feeding of suspected allergens have failed to withstand critical analysis. Radiologic examination of the gastrointestinal tract incorporating test materials in the barium meal has been proposed as an objective diagnostic aid in gastrointestinal allergy.[1] The chief changes observed have involved the motor function of the stomach and small intestine and have consisted of (1) hypertonicity and, less frequently, hypotonicity of the small bowel, (2) hypermotility as evidenced by a rapid transit of barium from the stomach to the cecum, (3) segmentation of the barium column, and (4) at times delayed gastric emptying. . These changes, however, are nonspecific and have been described is a variety of other disorders, including nutritional and purely psychogenic gastrointestinal ailments. The lack of specificity of these changes and the paucity of control studies have failed to inspire confidence in this technique. After a review of this field, Ingelfinger, Lowell, and Franklin[2] have suggested the following criteria for establishing the diagnosis of food allergy: "The food should be given in capsules or by stomach tube or in such a manner that the patient is unaware of its nature; reproducible symptoms, signs, or x-ray findings should consistently follow administration of the disguised food at a more or less constant interval, possibly not to exceed a few hours; other foods given to the patient in the same manner should not produce similar changes, and the suspected food given in the same manner to normal subjects should not cause the observed effects." The difficulty in fulfilling these criteria in clinical practice was recognized by these authors, who concluded that, "If the diagnosis could be firmly established in a number of cases, a characteristic pattern might become apparent and correlation with indirect tests might be possible."

An excellent and well-controlled study of the type just suggested was reported by Tallant, O'Neill, Urbach, and Price,[3] utilizing the x-ray examination of the small intestine as a diagnostic aid in a group of patients with suspected food allergy. They studied 12 patients with gastrointestinal food hypersensitivity and first subjected them to x-ray study of the small intestine with a mixture of barium and water as a control after at least 5 days without ingestion of the allergen. These studies were essentially normal. Four or 5 days later, the x-ray study was repeated using, however, barium plus the suspected allergen for the test meal. All patients exhibited striking motility

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changes in the small intestine. One patient underwent a third study using egg to which he was not sensitive, and his small intestinal motility was found again to be normal. Eight nonallergic persons were tested in a similar fashion using milk, egg, pork, and corn, all with negative findings. Five food allergic patients without gastrointestinal disturbances were similarly studied and although their allergic complaints (neurodermatitis, asthma, and angioneurotic edema) were provoked by the test foods, no changes in the motility of the small intestine were induced. This report clearly establishes the value of x-ray examination of the small bowel in the study of food allergy and should answer many of the criticisms offered in the past. Further support for the usefulness of this technique has been presented by Schloss,[4] who was able by Miller-Abbott balloon technique to confirm the production of motility disturbances by allergens introduced directly into the jejunum of 2 subjects whose x-ray studies had revealed striking motility changes after the feeding of the allergen.

In the present study, we have tried to evaluate the usefulness of the small intestine x-ray examination as an objective technique in 20 patients with suspected gastrointestinal food allergy. It was not possible to subject all of these patients to careful control studies but a few representative cases were so studied.

METHODS

X-ray studies of the small intestine were performed according to the technique described by Golden[1] using 4 ounces of barium sulfate in normal saline solution. Gastrointestinal motility was observed fluoroscopically and films were made at appropriate intervals. The features especially stressed were: (1) gastric emptying, (2) transit time of barium from the stomach to the cecum, (3) mucosal pattern, (4) tonicity (caliber of the lumen of the intestine), and (5) segmentation or lack of continuity of the barium column. Most of these patients were selected for this investigation because of histories suggesting food allergy with gastrointestinal manifestations. The latter consisted of flatulence, abdominal pain, and often diarrhea and nausea following the ingestion of specific foods. In 50 per cent of these cases, hives followed the ingestion of the offending allergen. Several patients exhibited marked eosinophilia but as a rule the eosinophil count was not elevated. A history of migraine was elicited in 7 cases. Four patients had experienced asthma. The commonest allergen was whole milk (70 per cent of the cases). Careful clinical and laboratory study excluded the presence of inflammatory, primary nutritional, parasitic, or neoplastic disease of the gastrointestinal tract in these patients.

RESULTS

The motility changes reported in the past[1, 2] as manifestations of food allergy were encountered in 18 of the patients studied. In 2 patients, despite a history strongly suggestive of food allergy, no significant change could be demonstrated in small bowel motor function. The changes observed consisted of (1) hypermotility with rapid transit of barium from the stomach to the cecum in 14 patients (hypomotility was encountered in 4 patients), (2) hypertonicity of the small intestine, especially the middle and lower portions, and

(3) segmentation or discontinuity of the barium column. At times gastric emptying was delayed, but this was not a constant finding. These changes could be provoked by administration of the allergen during the course of the x-ray study and were often accompanied by symptoms of abdominal distress, cramps, and diarrhea. Elimination of the offending foods resulted in significant and often dramatic clearing of symptoms. The administration of antihistaminic drugs, among them elixir of Benadryl, Pyribenzamine, Neoantergan, and Perazil, was helpful in the management of acute symptoms and in the treatment of patients whose multiple sensitivities precluded the use of an elimination diet for any prolonged period. In some patients it was possible to allow small amounts of allergen in the diet providing antihistaminic medication was given. In a few, it seemed that food allergy disappeared if the patient were allowed to continue to eat the offending food while taking antihistaminic medication to minimize symptoms.

Of considerable interest was the observation that many patients exhibited motility changes in the small intestine before the allergen was administered at the time of examination. These changes, when present, were accentuated by feeding the offending allergen. In the past, it has been assumed that those changes might well represent the carry-over from previously ingested allergen. However, another theory is that the small intestine motility changes may be manifestations of an intrinsic or constitutional state of irritability of the small intestine (probably related to acetylcholine liberation) rendering the small intestine of these individuals peculiarly susceptible to the stimulus of various cholinergic trigger mechanisms, for example, histamine, chemical, bacterial, mechanical irritants, or psychongenic disturbances. This concept would be in conformity with the nonspecificity of the small intestine x-ray changes and it would also aid in the understanding of the findings just recorded. The food allergic patient may have a constitutional or intrinsic disorder of gastrointestinal motility rendering him peculiarly susceptible to the effects of various cholinergic stimuli. This may vary in severity from day to day and may account for some of the vagaries of food allergy. The attribution of the small intestine x-ray changes to cholinergic phenomena may also be helpful in understanding why identical changes may appear in those patients under psychogenic stress and when offending foods are ingested. The identification and elimination of offending foods or sometimes the administration of antihistaminics will relieve the symptoms in this group of patients and this may help to differentiate them from the purely psychogenic cases.

In illustration of the preceding discussion there are presented below several representative protocols of individual cases.

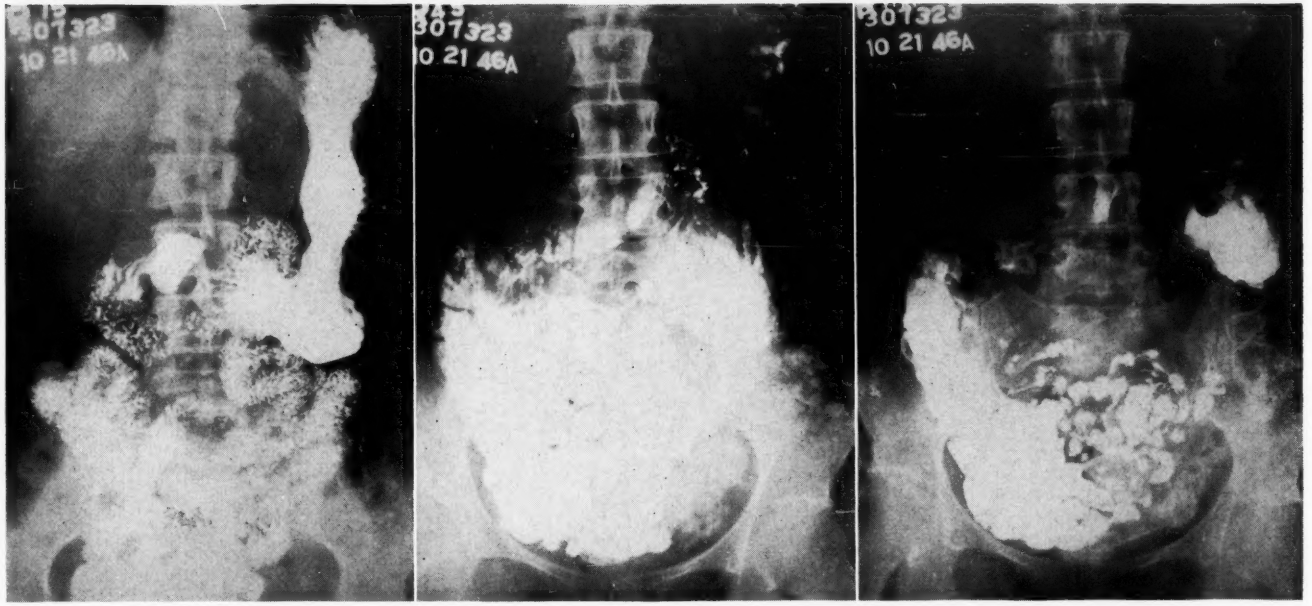
CASE 1.—A. M., a 41-year-old Cuban housewife, had for some years been troubled with flatulence, abdominal distress, and diarrhea following the ingestion of milk, eggs, or chocolate. She eliminated these foods from her diet and then, because of persistent symptoms, eliminated many others. When first seen by us in October, 1946, she presented the picture of severe malnutrition induced by a diet grossly deficient in proteins, calories, and minerals. The small intestine x-ray study on Oct. 16, 1946, revealed hypermotility and hypertonicity and segmentation of the mid-small intestinal region, interpreted as suggesting food allergy. On October 21, 1946, the x-ray study was repeated and again evidence was seen of hypermotility of the

small intestine, the barium suspension reaching the lower ileum within 15 minutes (Fig. 1). Fifteen minutes later, the patient was given an eggnog. This produced a violent reaction manifested by rapid propulsion of the meal into the sigmoid, markedly increased segmentation, and hypertonicity of the jejunum and ileum and diarrhea and abdominal cramps. Since it was evident from the clinical history that this patient was sensitive to a wide variety of foods, an elimination diet could not solve her problem, for adequate nutrition could not be maintained. She was consequently given elixir of Benadryl, 8 ml., before each meal and at bedtime, and encouraged to broaden her diet and to eliminate only the very worst offenders. During her hospital stay she was able, while taking Benadryl, to eat foods which in the past had produced much distress. She was examined again 1 year later, at which time she left much improved and had gained 20 pounds. She was still taking Benadryl and was able to tolerate most foods except milk, which caused diarrhea unless it had been boiled.

CASE 2.—E. Z., a 50-year-old chemist, complained of diarrhea of 2 months' duration. He had known for years that milk caused considerable abdominal distress and flatulence. He was subject to ragweed hay fever. Careful studies failed to reveal any organic lesion of the gastrointestinal tract. A small intestine x-ray study on Jan. 20, 1949, revealed slight hypomotility, the barium column entering the cecum at 4 hours. At this time a glass of milk was given to the patient and this had a considerable simulating effect upon the intestine and within 30 minutes the barium column had passed from the cecum to the sigmoid and the lower ileum had become hypertonic (Fig. 2). He was advised to take elixir of Benadryl, 8 ml. before each meal, and to eliminate milk. The diarrhea stopped within 24 hours and subsequently he stopped using Benadryl but continued to eliminate milk. He then was free of diarrhea except on 2 occasions which he was able to attribute to corn bread and rye bread; these he eliminated from his diet with good results.

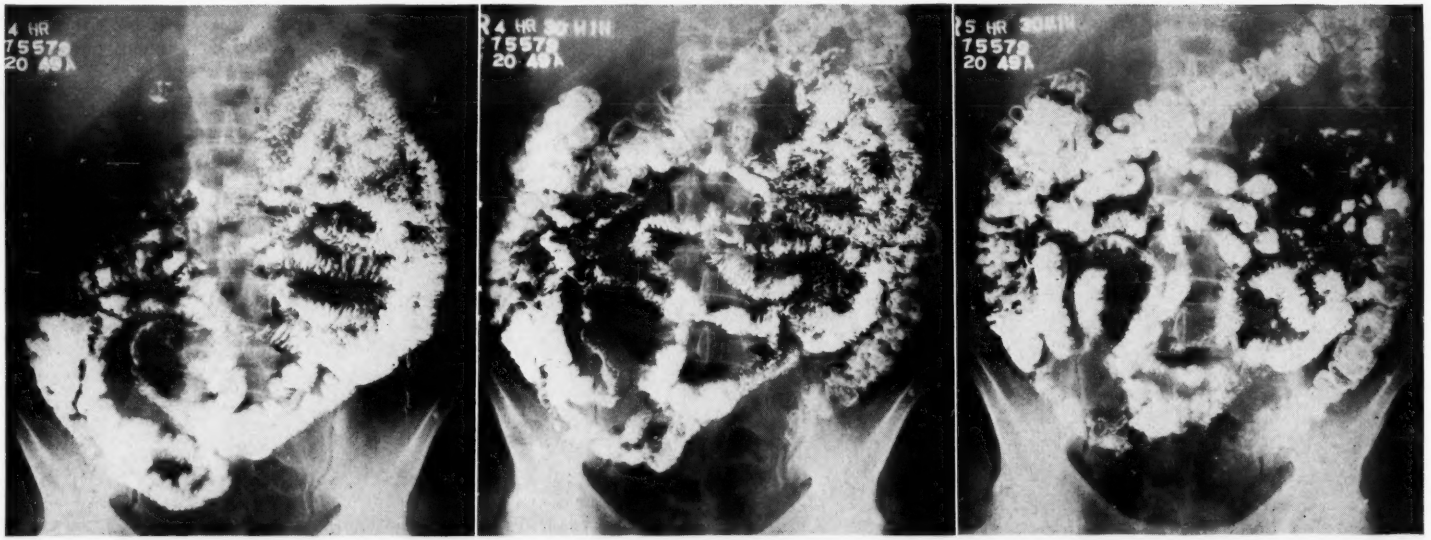
CASE 3.—D. G., a 40-year-old housewife, complained of abdominal distress, pain in the left lower quadrant, flatulence, abdominal cramps, and diarrhea, off and on for the past 7 years. During this time she had also developed hives and symptoms of allergic rhinitis. No organic cause for her symptoms had been discovered despite much study in several medical centers. She recalled that milk had always caused flatulence and at times diarrhea, so that she usually avoided it. On Dec. 9, 1948, a small intestine x-ray study revealed a slightly delayed transit time. However, after a glass of milk, considerable stimulation occurred, with rapid passage of the barium column into the transverse colon and marked hypertonicity of the mid-portion of the ileum. At the time of the examination, no symptoms occurred. However, about 7 hours later, the patient experienced a severe bout of abdominal distress, diarrhea, nausea and vomiting, and severe borborygmi. When seen by her local physician her blood pressure had fallen to 80/60. The episode cleared spontaneously within 48 hours and suggested to us a delayed allergic reaction to milk.

CASE 4.—T. Z., a 29-year-old clerk, complained of abdominal distress and diarrhea. During World War II he was stationed in the Pacific for 3 years and was unable to obtain whole milk and refused to drink reconstituted powdered milk. On returning to San Diego, Calif., in the fall of 1945, he was able to obtain a 5 gallon container of fresh milk daily for his outfit. He claimed he drank all of it himself, 5 gallons daily for 4 to 5 weeks! He gained 30 pounds and felt perfectly well. He was subsequently transferred to a post where milk was more difficult to obtain. Several months later, he was discharged and began work as a clerk in a grocery store. He drank 1 quart of milk while at work and developed abdominal cramps and diarrhea. The next day, the same thing occurred 4 hours after drinking milk. He consulted a physician who prescribed a low residue diet and advised him to continue the milk. However, the patient noticed that milk, even in small quantities, invariably produced abdominal cramps and diarrhea. The patient, therefore, decided to omit milk from his diet. However, he was still curious about this effect and decided to consult another physician, who also told him that milk could not be causing his symptoms and advised him to continue with it. However, the patient avoided milk until 1 day when he ate blueberries with a small amount of milk. One hour later he developed epigastric cramping pain followed by generalized abdominal distress and diarrhea which continued for 7 hours. He then consulted us and on Aug. 4, 1949,



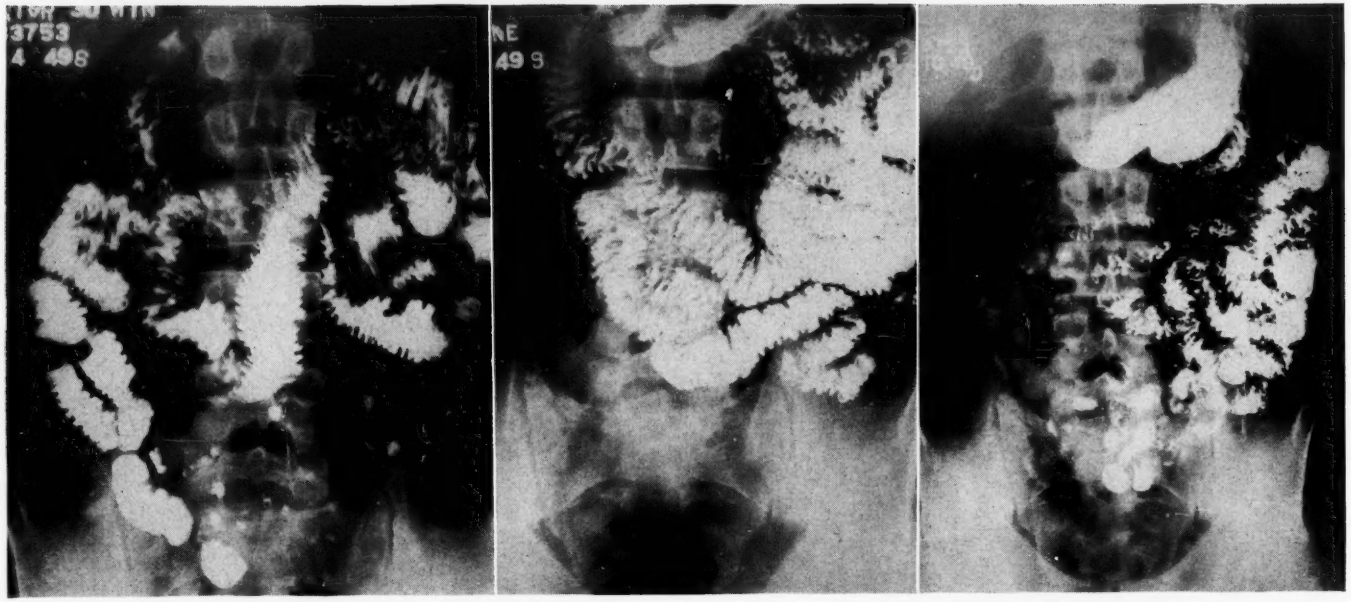
A. *B.* *C.*

Fig. 1 (Patient A. M.).—The illustration on the left is of the film taken 15 minutes after the ingestion of barium. Fifteen minutes later the patient was given an eggnog, which produced a violent reaction with rapid propulsion of the barium meal into the sigmoid, markedly widened segmentation, and hypertonicity of the jejunum and ileum as demonstrated in the center film, taken 15 minutes after the eggnog, and the film on the right, taken 30 minutes after the eggnog.



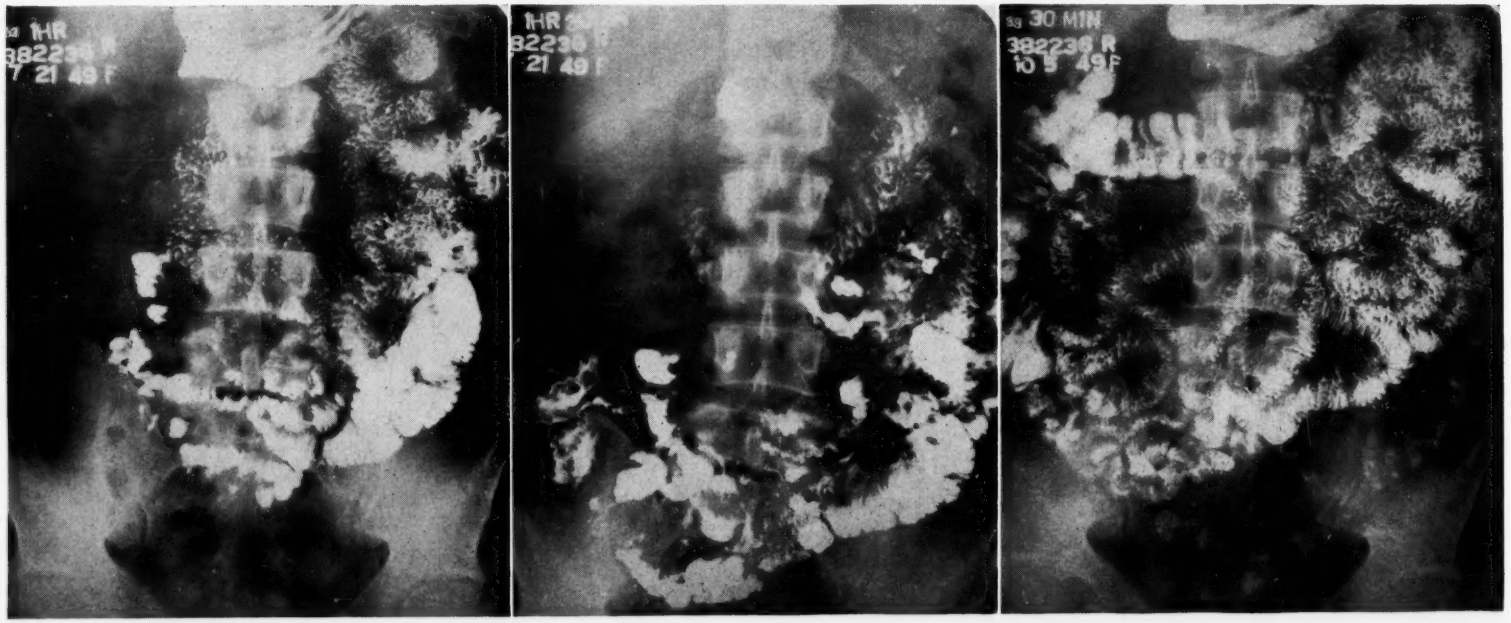
A. *B.* *C.*

Fig. 2 (Patient E. Z.).—The view on the left is at 4 hours after taking barium. Following it, milk was given by mouth, and it had a considerable stimulating effect on the intestine. Within 30 minutes, as seen in the center and right-hand illustrations, the barium column had passed from the cecum to the sigmoid and the lower ileum had become hypertonic.



A. *B.* *C.*

Fig. 3 (Patient T. Z.).—The illustration on the left is the 1½ hour film of the study of Aug. 4, 1949, of this milk-allergic patient; it reveals a disturbance in motor physiology of the small intestine with widening of the folds of the jejunum and slight segmentation. The center view is the 1 hour film of the study of Oct. 26, 1949, after the patient had eliminated milk for 11 weeks and was symptom-free. The small bowel now appears normal. On Nov. 10, 1949, a repeat x-ray study was done, and at this time milk was mixed with the barium meal. The illustration on the right is the 1 hour film of that study and again there is evidence of small bowel motor dysfunction provoked by milk.



A *B* *C*

Fig. 4 (Patient C. K.).—Ulcerative colitis and milk allergy. The illustration on the left is the 1 hour small intestine film taken July 21, 1949, revealing a disturbance in motor function of the small intestine; this is accentuated in the center view of the 1½ hour film. The patient was taken off milk. The repeat study taken on Oct. 5, 1949, seen on the right reveals in the 30 minute film an essentially normal small intestine with the exception of hypermotility.

a small intestine x-ray study was performed and it revealed a moderate disturbance in motor physiology of the small intestine manifested by widening of the mucosal folds in the jejunum and slight segmentation. There was, however, no appreciable immediate reaction to milk. The patient was placed on a milk-free diet and returned for re-examination of the small intestine on Oct. 26, 1949, at which time evidence of considerable improvement was found in the small intestine motor physiology (Fig. 3). The patient has avoided milk and has been free of symptoms up to the present.

In some of our patients the diagnosis of food allergy was entertained only after the motility of the small intestine had been reported as abnormal by the radiologist. Follow-up study of the patients was especially interesting, for it often revealed a previously unrecorded history of food sensitivity.

This is illustrated by the case of J. N., a 13-year-old feeble-minded boy who was brought to the clinic by his mother for investigation of diarrhea which had been present for some years. An adequate history was almost impossible to obtain because of the patient's mental retardation and his mother's low level of intelligence. A small intestine x-ray study revealed very striking changes in motility, present to a mild degree before giving milk and aggravated remarkably by the ingestion of milk. After this report was obtained, the history was checked with the patient's mother, who recalled that this child had always been different from her other children because milk had always "given him the runs." Without the x-ray study of the small intestine, the diagnosis would probably not have been suspected in this patient.

While the recognition of food allergy is of considerable importance in patients with uncomplicated gastrointestinal disturbances, it is of even greater significance in the patient with organic gastrointestinal disease to which it may be contributing. This is illustrated by the following protocol of a patient with nonspecific ulcerative colitis.

C. K., a 32-year-old merchant seaman, developed diarrhea and abdominal cramps in October, 1948, for which he was treated in the Marine Hospital, Staten Island, for 2 months, and the diarrhea subsided. In May, 1949, he had a recurrence of bloody diarrhea during which he lost 35 pounds. At this time he was living in a hotel room and subsisting on a diet consisting chiefly of milk despite the fact that milk had always caused him to have diarrhea in the past. Sigmoidoscopy on July 5, 1949, revealed severe and classical ulcerative colitis. A small intestine x-ray study revealed hypermotility, segmentation, and hypertonicity present to some degree before giving milk but markedly accentuated after giving milk (Fig. 4). Eosinophilia of 32 per cent was found but the remainder of the laboratory studies were negative. He was advised to stop milk and was given Neoantergan, 50 mg., before each meal and at bedtime. Within 24 hours the diarrhea had begun to lessen, and within 72 hours he was having semiformed stools. Improvement was dramatic and he quickly regained weight. His clinical improvement was accompanied by sigmoidoscopic improvement and within 1 month he was in a complete clinical remission.

He was seen occasionally and in March, 1950, he reported that after feeling perfectly well and gaining 30 pounds he had become involved in a violent family quarrel 3 weeks before and had almost immediately developed a recurrence of bloody diarrhea. He had eliminated milk and tried Neoantergan to no avail and had lost 36 pounds in 3 weeks. Again the sigmoidoscopic findings were those of acute severe nonspecific ulcerative colitis. He again had an eosinophilia of 19 per cent. Treatment consisted of Banthine,[5]* an anticholinergic drug which has a selective action on the gastrointestinal tract. This induced a dramatic remission within 24 to 48 hours and this was well sustained so that within 5 weeks he had regained almost 10 pounds and was having 1 formed bowel movement daily without blood or mucus. The eosinophil count dropped gradually from 19 to 14 to 10 to 1 per cent by the fourth week of treatment.

*Banthine was generously supplied by G. D. Searle & Co., Chicago, Ill.

For some years Andreson[6] has suggested that food allergy is responsible to a large degree for the illness called ulcerative colitis. The case just described could support this belief. However, in our experience, the striking sequence of events just listed has been the exception rather than the rule. It is of interest that in this patient the initial episode of colitis treated by us responded dramatically to an elimination diet and an antihistaminic. The second episode appeared to have been initiated by an emotional upset rather than an allergic stimulus and curiously enough, this attack was also accompanied by a high eosinophil count. Apparently a psychogenic cholinergic stimulus had reproduced the picture which 1 year before had been initiated by milk allergy. The beneficial effect of Banthine, an anticholinergic drug, was so striking that further investigation of this phenomenon has been initiated and will be the subject of a later report.[7]

SUMMARY

The x-ray examination of the small intestine in patients with food allergy affords an objective method which is useful in the study of this disorder. The motility changes are nonspecific and are found in a variety of conditions. They may, however, be modified in many instances by the withdrawal of allergenic foods or by the administration of antihistaminic drugs and by this means a degree of specificity may be achieved. The nonspecificity of these changes is comprehensible if they are viewed as results of cholinergic stimulation of an unstable or irritable autonomic nervous system. This may well be the common denominator in the response of the small intestine to allergens, mechanical, chemical, and bacterial irritants, and psychogenic threats. Further study is being made of this important point and this includes the further investigation of anticholinergic drugs such as Banthine.

The role of food allergy in the causation or aggravation of organic disease of the intestine, stressed by many and deprecated by others, has suffered because of the lack of objective methods of study. By the use of the x-ray examination of the small intestine, giving the suspected allergen in the barium meal, or during the course of the study, it has been possible to produce marked disturbances of motility in food-sensitive persons, in support of these clinical histories. This technique may be of considerable importance in the study of some patients with ulcerative colitis and other organic diseases of the gastrointestinal tract.

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STUDIES IN BACTERIAL ALLERGY*†

IV. The Transitory Nature of Desensitization of the Passively Sensitized Guinea Pig Uterus With a Bacterial Hapten, With a Note on 95 Per Cent O_2 - 5 Per Cent CO_2 as an Aerating Mixture

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DESENSITIZATION of actively sensitized guinea pigs with whole antigens is transient,[1] probably because the desensitizing antigen stimulates further anaphylactic antibody formation.

The uterine horns of guinea pigs are reported to be desensitized by whole antigens.[2-5] The duration of desensitization has not been studied.

The present experiments were planned to study the role of haptens in the desensitization of uterine horns of passively sensitized guinea pigs. Pneumococcal polysaccharides were selected as representative bacterial haptens because of their limited functions as antigens.[6, 7]

Spontaneous resensitization of the desensitized uterine horn was observed, unexpectedly. This phenomenon, which has not been described before, is the main point of interest in this report. The relation between spontaneous resensitization and Dale's[4] supposed in vitro passive sensitization of excised uterine horns will be discussed briefly.

METHODS

Small virgin guinea pigs were sensitized passively with 0.2 to 0.5 ml. of refined antipneumococcal rabbit serum,§ given intra-abdominally. They were killed by a blow on the head 24 to 72 hours later. The uterine horns were excised, suspended[4] in 125 ml. of Tyrode's[8] solution at 37° C., and aerated with a 95 per cent oxygen-5 per cent carbon dioxide mixture. When 0.2 mg. of type-specific polysaccharide‖ was added to the Tyrode's bath, a marked contraction occurred in about two-thirds of the horns. Those that did not contract were discarded. Nonspecific polysaccharides were used as controls. Types I, II, and IV pneumococcal polysaccharides and antisera were used.

DESENSITIZATION

Two methods were used: (1) contracted horns were left in the hapten baths until they relaxed to their original lengths[3]; and (2) contracted horns were allowed to relax in fresh Tyrode baths. They were then re-exposed to

*These studies were made possible in part by a grant from Eli Lilly & Company, Indianapolis, Ind.

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§Generously supplied by Dr. B. Scott Fritz of the Marietta Laboratories, Wyeth, Incorporated, Marietta, Pa. Types I and II antisera contained 4,000 units per milliliter. Type IV contained about 6,600 units per milliliter.

‖Generously supplied by Dr. H. D. Piersma of the Lederle Laboratories, Pearl River, N. Y.

the hapten bath. This alternation between hapten and fresh bath was continued until there was no further reaction to the hapten. Horns desensitized by either method still gave strong reactions to histamine. Two of 3 horns passively sensitized to 2 types of pneumococci, when desensitized with one type-specific hapten, remained fully sensitive to the other.

Nonspecific polysaccharide control haptens did not elicit contractions at any time.

RETURN OF SENSITIVITY

After desensitization, the horns were flushed with Tyrode's solution and placed in fresh baths. The horns were rechallenged with 0.2 mg. polysaccharide after varying intervals. Table I shows that none of the horns reacted after 10 minutes in the fresh bath. Eighteen of 19 horns showed full contractions when tested after 4 or more hours in the baths. In sharp contrast, only one horn showed a full contraction after less than 4 hours in the bath.

TIME ELAPSED IN PARTIAL AND COMPLETE SPONTANEOUS RESENSITIZATION OF 26 DESENSITIZED UTERINE HORNS OF GUINEA PIGS

RETURN OF SENSITIZATION	INTERVAL BEFORE RETEST														
	MINUTES	HOURS													
	10	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Partial		1	1	2	1										
Complete				1	9	4		1					2		2
None	26	2													

The method of desensitization did not affect the return of sensitization.

When partially resensitized horns (less than 4 hours in the fresh bath, Table I) were again desensitized, they became fully resensitized after 4 or more hours in a second fresh bath.

Successive desensitization and resensitization was demonstrated 2 or more times in several horns.

Every horn which was desensitized exhibited the phenomenon of resensitization one or more times. The return of sensitivity was not impeded in the 2 instances in which the horns were left in the hapten bath for one hour after desensitization. The horns were challenged regularly with nonspecific polysaccharide controls. The return of sensitivity was always specific.

Fig. 1 illustrates a typical sequence of events.

DISCUSSION

The data presented confirm reports[9] that specific pneumococcal polysaccharide will cause contraction and desensitization of uterine horns of passively sensitized guinea pigs. The data further show that this desensitization is transient. Resensitization is complete in 4 hours. It is demonstrable in lesser degree in from 1 to 3 hours. This is in contrast to implications[2-5] that uterine horns are thus permanently desensitized to a given concentration of antigen.

Dale[4] found that uterine horns specifically desensitized with horse serum became partially, but not completely, resensitized after 2½ hours in anti-horse guinea pig serum. His desensitized horns, after a similar interval in Ringer's

solution, showed no spontaneous resensitization. The only desensitized control horn tested for resensitization after more than 2½ hours in Ringer's solution did not react. Dale interpreted these experiments to show passive sensitization in vitro. Inexplicably (to him), he found that normal uteri, after 2½ hours in the anti-horse guinea pig serum, were not sensitive. The hypothesis is tenable, therefore, that Dale was dealing with this phenomenon of spontaneous resensitization instead of, as he supposed, with in vitro passive sensitization of the excised uterine horn of the guinea pig.

Experimental explanation of this spontaneous resensitization has not been attempted. Antigen-antibody combinations (precipitates, and so on) have been reversed by heat,[10] alkali,[11] salts,[10-12] and excess of antigen.[13] These conditions are not present in the Tyrode bath.

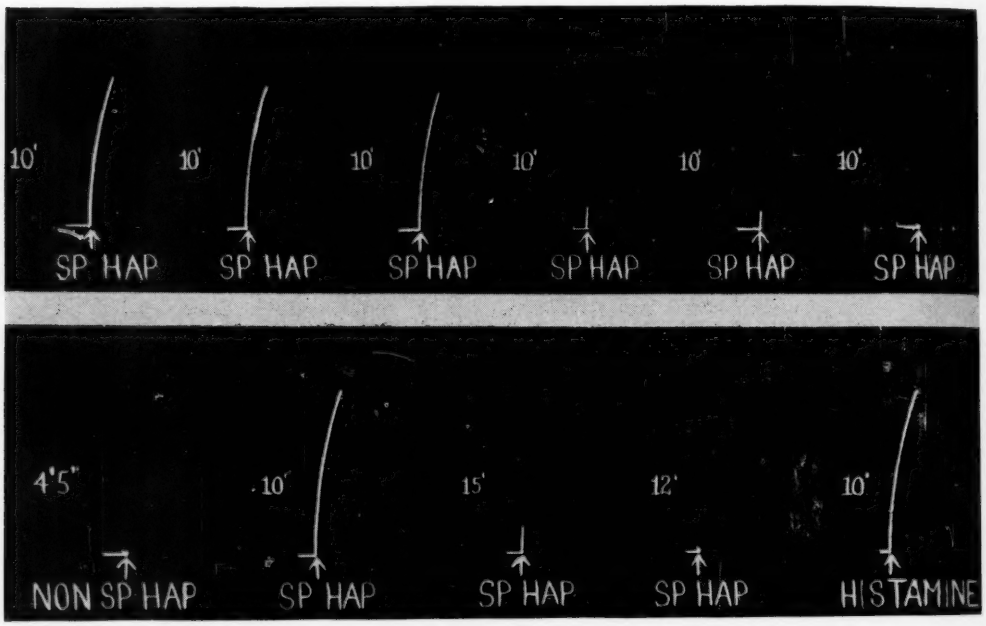

Fig. 1.—Illustration of specific desensitization (Method 2), spontaneous resensitization, and re-desensitization. Sp. Hap. = specific hapten; Non Sp. Hap. = nonspecific hapten.

Top row: 3 full and 2 slight specific contractions, at 10 minute intervals, followed by desensitization.

The horn relaxed in fresh Tyrode's bath after each reaction to hapten.

Bottom row, after 4 hours and 5 minutes in Tyrode's solution: no reaction to nonspecific hapten, full response to specific hapten, slight specific contraction, desensitization, full response to histamine.

One can postulate that the hapten-antibody combination is less firm than the antibody-tissue union. Sufficient hapten could thus be released to the bath in 4 hours to free enough antibody-tissue combinations to react fully with new hapten. This should be susceptible to proof.

The experiments were handicapped initially by the development of hyperreactivity of the excised horns, followed by their death. This was traced to a relatively rapid increase in the pH of the Tyrode solution when compressed air

was used to aerate the bath. When a 95 per cent O_2 - 5 per cent CO_2 mixture was substituted, the approximate pH 7.4 was maintained for hours. Thereafter, all initially sensitive horns maintained an almost uniform reactivity for the duration of the individual experiments (Table I). No effort was made to see how much longer the horns would remain reactive. Sustained allergic reactivity, plus the phenomenon of resensitization, should increase the usefulness of excised uterine horns as experimental tools.

CONCLUSIONS

1. Pneumococcal polysaccharides, specifically, elicit contractions of, and desensitize, excised uterine horns of passively sensitized guinea pigs.

2. After 4 or more hours in Tyrode's solution the uterine horns become fully resensitized and react fully to the readdition of the original concentration of the specific pneumococcal polysaccharide. In other words, desensitization of uterine horns, as described, is transient.

3. This sequence of desensitization and spontaneous resensitization was repeated 2 or more times in the same horn.

4. It is believed that Dale's observation of the reappearance of sensitivity in uterine horns in immune serum baths may have been due, in part, to spontaneous resensitization.

5. Sustained specific allergic reactivity for 12 hours or longer in Tyrode's bath aerated with 95 per cent O_2 - 5 per cent CO_2, plus the phenomenon of spontaneous resensitization after desensitization should increase the usefulness of excised uterine horns as experimental tools.

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STUDIES ON THE MECHANISM OF FATAL ANAPHYLAXIS IN THE RABBIT*†

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INTRODUCTION

SYSTEMIC anaphylaxis in the rabbit has been reported to be characterized by pathologic-physiologic changes within the cardiovascular system.[1-4] Rabbits dying in anaphylactic shock exhibit extreme dilatation of the right auricle and ventricle, congestion of the liver, and distention of the portal vein and inferior vena cava. Airila[1] first noted the rise in pulmonary arterial pressure after the shocking injection and the fall in systemic blood pressure as soon as the high pulmonary pressure developed. Coca[2] stated that the altered hemodynamics in rabbit anaphylaxis resulted from contraction of the smooth musculature of the pulmonary arterioles leading eventually to obstruction of flow through the constricted lumen of the involved vessels. The pulmonary arterial bed is thus practically occluded and dilatation of the right side of the heart follows. Drinker and Bronfenbrenner,[3] by direct pressure readings from the exposed pulmonary artery, recorded the rise in pressure in the pulmonary circulation and fall in systemic arterial pressure during anaphylactic shock, and by means of the Starling heart preparation then demonstrated that the heart does not suffer primary damage in anaphylaxis but is secondarily affected due to its inability to maintain the necessary pressure head to force blood through the pulmonary vessels. The experiments of Friedberger and Seidenberg[5] demonstrated resistance to flow of antigen-containing perfusion fluid through the lung of the sensitized animal, and Grove[6] noted constriction of isolated strips of the pulmonary artery in the Dale bath. Vallery-Radot, Halpern, and Mauric[4] concluded that pulmonary vasoconstriction is not the only cause of a drop in systemic blood pressure, for in their experience the generalized drop in arterial blood pressure and the rise in pressure in the pulmonary artery seem to occur independently.

Auer and Robinson[7] reported electrocardiographic disturbances in conduction in the heart of the rabbit during anaphylaxis which ranged from slight delay to partial and complete heart block. Criep[8] studied cardiac changes, as revealed by the electrocardiogram, in the rabbit during anaphylaxis, asphyxia, and mechanical clamping of the pulmonary artery. In all 3 states there were noted identical changes consisting of the development of conduction disturbances progressing to cardiac standstill. Criep indicated that the cardiac changes observed were due to myocardial anoxemia and possibly cerebral anoxemia secondary to the state of asphyxia induced by anaphylaxis.

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†This project was aided in part by a grant from the Sarah Mellon Scaife Foundation.

This report deals with our attempt to study the pathologic physiology of anaphylaxis in the rabbit by electrocardiographic and pneumographic studies and by recording intracardiac pressure during anaphylactic shock.

EXPERIMENTAL PROCEDURE

The techniques of sensitization and subsequent challenge in eliciting anaphylaxis in the rabbit were carried out according to the conditions suggested by Bukantz, Johnson, and Alexander.[9] Adult rabbits ranging in weight from 2.5 kg. to 3.5 kg. were sensitized by a single intramuscular injection of 325 mg. of bovine gamma globulin (Fraction II, Armour) absorbed on aluminum hydroxide prepared according to the method of Hektoen and Welker.[10] Three to 4 weeks later fatal anaphylactic shock was induced by the intravenous administration of 500 mg. of bovine serum gamma globulin in 10 ml. of physiologic saline.

Just prior to the administration of the shocking dose of antigen, 6 sensitized animals were lightly anesthetized with intravenously administered pentobarbital sodium. This degree of anesthesia facilitated the procedures required in exposing the jugular vein and passing a catheter into the heart, but at the same time did not interfere with respiration, cardiac action, the production of anaphylaxis, or the symptoms occurring during anaphylactic shock.

Blood pressures within the right ventricle were obtained with a No. 6 ureteral catheter introduced through the exposed right jugular vein and attached to a Statham P23A pressure transducer. Recordings on a constant speed kymograph were obtained utilizing a direct coupled high gain stabilized amplifier which drove an Edin pen motor. Calibration of the transducer was obtained by standardization against a mercury column, as well as by the integral electrical calibration. Heparinized saline solution was used to fill the transducer and catheter system. Electrocardiograms employing the standard Lead II and respirations utilizing a pneumatic system were also simultaneously recorded by pen writing arms on the constant speed kymograph. Two additional rabbits were similarly studied except that pulse pressure rather than blood pressure was recorded.

Following death, the thoracic cage of each animal was opened, the heart grossly examined, and the position of the catheter checked.

RESULTS

The method of Bukantz, Johnson, and Alexander[9] for sensitizing and challenging rabbits, which was employed in this experiment, was uniformly successful in effecting fatal anaphylactic shock in the animals studied.

The effects of fatal anaphylaxis on respiration, electrocardiographic tracings and right ventricular pressure are summarized in Table I.

In 6 rabbits there was an immediate rise in both systolic and diastolic pressure within the right ventricle reaching a maximum within 20 to 60 seconds following the administration of the shocking dose of antigen. The variations in intraventricular pressure noted at the height of anaphylaxis were related to the convulsive seizures and the marked fluctuations in respiratory movements that occurred. After the rise of intraventricular pressure to a maximum height,

TABLE I. CHANGES IN RIGHT INTRAVENTRICULAR PRESSURES, RESPIRATIONS, AND ELECTROCARDIOGRAPH TRACINGS DURING ANAPHYLAXIS IN SENSITIZED ANIMALS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF A SHOCKING DOSE OF ANTIGEN

RABBIT NUMBER	CONTROL INTRAVENTRICULAR PRESSURES (MM. HG)		INTRAVENTRICULAR PRESSURES AFTER INJECTION OF ANTIGEN (MM. HG)		TIME OF APPEARANCE OF MAXIMUM VENTRICULAR PRESSURE FOLLOWING INJECTION OF ANTIGEN (SECONDS)	TIME OF CESSATION OF RESPIRATION FOLLOWING INJECTION OF ANTIGEN (SECONDS)	DURATION OF TIME FROM ADMINISTRATION OF ANTIGEN UNTIL DISAPPEARANCE OF INTRAVENTRICULAR PRESSURES (MINUTES)	ELECTROCARDIOGRAM
	SYSTOLIC	DIASTOLIC	SYSTOLIC	DIASTOLIC				
1	9-10.5	4-4.5	13.5-16.5	7.5-9	20	80	8	Cardiac slowing and prolonged QRS after 2 min.
2	6.6-9.9	2.2-4.4	16.5-38.5	6.6-8.8	20	120	2	No change
3	7.7-9.9	1.1-3.6	8.8-38.5	2.2-6.6	20	70	9	Marked lowering of ST segment; otherwise not significant
4	9.6-12.8	1.8-4.8	16-17.6	8-9.6	50	130	6	No change
5	10-12.8	1-1.8	17.2-19.8	3.6-7.2	60	120	10	No change
6	12-18	3-.7	16-20	6.8	60	60	4	No change

both the systolic and diastolic pressures gradually fell to zero in from 2 to 10 minutes following the intravenous administration of the antigen.

With the exception of a few terminal gasps, respirations abruptly ceased within 60 to 120 seconds after the administration of the antigen. In 1 rabbit (No. 2) respirations and intraventricular pressure changes ceased simultaneously after 2 minutes. However, in the other 5 rabbits studied, pulsatile changes in intracardiac pressure continued for a period of from 2 to 10 minutes after respirations had ceased (Fig. 1). In 2 additional sensitized rabbits, similarly studied, in which pulse pressures rather than true blood pressures were recorded, the fatal reaction to the administration of antigen was also that of a sudden respiratory arrest occurring well before the disappearance of intraventricular pressure changes.

No significant changes in the electrocardiograms were noted during anaphylactic shock. All of the major electrocardiographic changes recorded occurred after respiration had ceased and the intraventricular pressure had fallen to zero and pulsatile variations no longer occurred.

In all instances, on post-mortem inspection, the tip of the catheter was found lying inside the right ventricle. Both the right auricle and ventricle felt flabby and were enlarged to approximately 3 times their normal size.

DISCUSSION

It is recognized that the administration of certain drugs may interfere with the production of typical anaphylaxis in experimental animals. However,

Drinker and Bronfenbrenner[3] did not find that the anesthesia employed in their studies interferred with the production of anaphylactic shock in rabbits, and Chase[11] was of the opinion that anesthetics are of low order in this regard. The effect of the anesthesia itself, together with the operative procedures involved, probably exerted no significant influence on the response of the animals to the challange dose of antigen, since similar fatal anaphylactic shock was elicited in unanesthetized sensitized rabbits, within similar time periods, employing the same doses of antigen.

The cause of death in anaphylactic shock in the rabbit has been considered to be attributable to acute failure of the right side of the heart secondary to obstruction of blood flow in the pulmonary circuit. Further evidence for the development of right-sided heart failure is noted in this study in that increased systolic and diastolic pressures were observed to develop within the right ventricle. Though the intracardiac pressures gradually diminished to zero indicating a failing heart, it is noteworthy that in 7 of the 8 rabbits studied in this experiment respirations suddenly ceased while cardiac function as measured

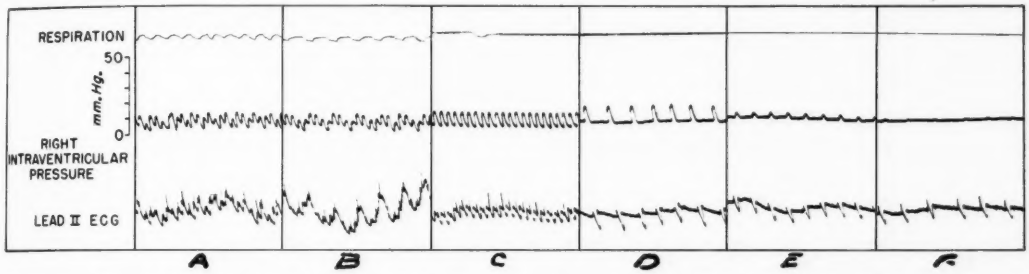

Fig. 1 (Rabbit No. 4).—Showing simultaneous recordings of respirations, right intraventricular pressures, and electrocardiographic tracing: *A*, during control period; *B*, during period of injection of shocking dose of antigen; *C*, 2 minutes later, showing abrupt cessation of respiration while the intraventricular pressures have risen and the electrocardiographic tracing is maintained; *D*, 2 minutes after respirations have ceased, increased intraventricular pressures and electrocardiographic tracing continue; *E*, 3 minutes after respirations have ceased, intraventricular pressures are diminishing to levels below normal; *F*, 4 minutes after the cessation of respiration, the intraventricular pressure has fallen to zero, the electrocardiographic tracing continues.

by right intraventricular pressure and electrocardiogram was maintained. The heart continued to beat and significant intraventricular pressures were recorded for several minutes following the cessation of respiration. The abrupt manner in which respirations stopped following the administration of the shocking dose of antigen suggests a sudden peripheral respiratory failure. The few terminal gasps that followed later were probably due to central anoxia. Continued beating of the heart following death has been previously noted in anaphylactic shock in the rabbit[12]; however, fatal anaphylaxis in the animal has always been attributed to acute congestive heart failure. Evidence of bronchospasm has not been found[11] and histologic study of the bronchioles and lung parenchyma showed no significant changes. Simmonds[13] explained the absence of bronchospasm on the assumption that bronchial muscles in the rabbit are relatively too weak to be capable, by contraction, of interfering with respiration. Grove[6] was unable to secure contraction of an isolated bronchial strip taken from a sensitized rabbit and immersed in a Dale bath with antigen added, but noted that this might indicate that the muscular tissue in the strip was so weak that its con-

traction would not be registered by that technique. The fact that major electrocardiographic changes did not occur until after respirations had ceased and intracardiac pressure changes were no longer recorded indicated that death was not due to disturbances in the conduction mechanism of the heart or myocardial damage that could be recorded by the electrocardiogram. The persistence of heart action for as long as 10 minutes following apparent peripheral respiratory failure suggests that the mechanism of death in fatal anaphylaxis in the rabbit is respiratory failure rather than acute failure of the right side of the heart.

SUMMARY

1. Right ventricular pressures and pneumographic and electrocardiographic tracings were recorded in sensitized rabbits during anaphylactic shock.

2. Following the intravenous administration of the shocking dose of antigen a rise in both systolic and diastolic right ventricular pressures was observed, followed by gradual reduction in pressure to zero.

3. In 7 of 8 rabbits studied, respirations ceased abruptly although the heart action and significant intracardiac pressure pulsations continued for as long as 10 minutes. In one animal heart action and respirations ceased simultaneously.

4. Major electrocardiographic changes did not occur until after respirations had ceased and the intraventricular pressure had fallen to zero.

5. It is suggested that the mode of death in anaphylaxis in rabbits is due, at least in part, to peripheral respiratory failure.

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THE ISOLATION OF ISOQUERCITRIN FROM GIANT RAGWEED POLLEN; THE ELECTROPHORETIC PATTERN AND BIOLOGIC ACTIVITY OF THE PIGMENT*

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IN THE course of studies of fractions of giant ragweed extracts, an amorphous precipitate was observed to accumulate if the solutions stood in the refrigerator for a length of time. A crystalline material obtained from this precipitate was analyzed and identified. Then the electrophoretic pattern of this isolated substance was compared with that of normal ragweed extracts. The study was completed by observing the skin reactions with the crude precipitate and the refined crystals in ragweed-sensitive persons.

When the amorphous precipitate recovered from the crude extracts was refined, the yellow needles obtained were found to be isoquercitrin. This glycoside is the 3-glucoside of quercetin, a pentahydroxy-flavone which is one of the most widely distributed natural pigments.[1] It occurs as quercetin in many plants, such as dyers oak, sumac, hops, and red roses, and in glycosidic forms, such as isoquercitrin, which are found less frequently. In the past flavones have been detected in pollens, but the identity of the pigmented derivative has not been clearly established. Quercetin, the hydroxy flavone, may be combined with rhamnose, galactose, or glucose. In isoquercitrin it is combined with glucose. The identity of the glucoside isolated was determined from an analysis for C, H, and O, from its molecular weight, by the isolation from hydrolyzates of quercetin and glucose, and by characteristic melting points.[1]

EXPERIMENTAL

Separation of the Precipitate.—The extracts yielding the most voluminous precipitates were made with ether-washed pollen and cold distilled water. Customarily 100 Gm. of giant ragweed pollen were divided into 4 equal portions which were washed successively with 4 lots of the smallest possible volume of distilled water. When these 4 lots were combined and filtered the total volume was about 300 c.c. Such extracts contained from 700 to 800 mg. of nitrogen and from 18 to 19 Gm. of solids determined by drying in vacuo. The pH of the solutions from most samples of ragweed was 5.6 to 5.7. When one of these ragweed extracts was refrigerated a cloud of amorphous precipitate appeared after several days and continued to aggregate and settle for 3 or 4 months. Six to 7 per cent of the 18 or 19 Gm. of solids in a satisfactory solution was carried down as precipitate. The precipitate washed once in a large volume of water and dried in vacuo contained from 0.5 to 0.7 per cent of nitrogen. The unmodified pH of the pollen extracted in water was most satisfactory for the separation as only

We are indebted to Wyeth, Incorporated, for the pollen used in these experiments.

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meager precipitation occurred in more alkaline (pH 6.5 to 7.5) or less concentrated solutions, and more acid solutions (pH 3.0 to 5.0) yielded precipitates with higher nitrogen contents.

Analysis of the Precipitate.—The crude precipitate was dissolved in hot ethanol and filtered. Then the fine yellow needles crystallizing from this filtrate were reprecipitated 4 times from boiling water. These needles were insoluble in cold water, soluble in alkalies and alcohol, reduced copper solutions after hydrolysis, and turned green in weak solution on the addition of ferric chloride. The color of a weak solution varied with the pH; it was colorless at 2.0, yellow 4.0 to 6.0, orange 6.5 to 7.5, and green above 8.5. The crystals contained no nitrogen, as determined by micro-Kjeldahl both by titration and colorimetrically, and gave no ash. Duplicate analyses of samples dried at 110° C. were as follows: C, 54.2 to 54.34; H, 4.19 to 4.24; calculated O, 41.5. Samples dried at 75° melted at 214° to 216° with decomposition. From these observations and analyses together with subsequent data the isolated material was believed to be isoquercitrin, a tetrahydroxyflavone[3] glucoside (C_{21}, H_{20}, O_{12}). In the following data the theoretical values have been given in parentheses. Fifty milligrams of the dried crystals on hydrolysis in 5 ml. of 10 per cent HCl yielded 33 mg. of solid (theoretical 32.6 mg. calculated as quercetin). A second hydrolysis of 73 mg. in 7 ml. of 10 per cent HCl yielded 46 mg. of solid (theoretical yield of quercetin 47.5 mg.). The dried solid from this hydrolysis melted with decomposition at 312 to 314°. The precipitate from this hydrolysis, believed to be quercetin, was acetylated. Thirty-one milligrams of the precipitate, 30 mg. of anhydrous sodium acetate, and 3 ml. of acetic anhydride were heated on the steam bath for 1.5 hours. After cooling and pouring on ice a white precipitate formed: recrystallization from alcohol produced needles (37 mg.) with a melting point of 192 to 194°. The optical rotation of the supernatant solution from the hydrolysis of the 73 mg. sample was 51.5 (calculated $[\alpha]_D$ 52.8 for 1 mole of glucose per mole of glucoside).

Cutaneous Tests.—Patients strongly reactive to ragweed were tested with solutions of the crystallized isoquercitrin in physiologic salt solution titrated to pH 8.0 with bicarbonate. From data obtained in separating the crude precipitate it was determined that the approximate ratio of nitrogen to isoquercitrin in the ragweed extract was 7 to 11 mg. The solutions of the extract used to test the sensitivity of the patients to ragweed contained .001 and .0001 mg. of nitrogen per c.c. The reactions to these dilutions were compared with those obtained with solutions of the isoquercitrin made to correspond with these concentrations of nitrogen, that is, containing respectively .0016 and .00016 mg. of isoquercitrin. Although the patients tested were extremely sensitive to ragweed no immediate reactions were obtained with the glucoside, but delayed swelling and inflammation were observed the following day if solutions of the glucoside more concentrated than those indicated above were injected. No reactions were obtained with control solutions.

The isoquercitrin used in the preceding tests had been treated drastically with hot alcohol and boiling water. Less drastic methods would not free the

precipitate from nitrogen although the content could be reduced by acid precipitation and resolution of the precipitate by the gradual addition of bicarbonate. For example, a sample containing .67 per cent N was reduced to .36 per cent N by 4 precipitations and subsequently to .15 per cent but at a tremendous loss of material. This sample, which had never been heated and only subjected to a varying pH (4.0 to 8.2), was used for testing. Solutions (0.1 per cent) of the glucoside containing .00036 and .00015 nitrogen were compared with solutions of the original ragweed extract containing .001 and .0001 per cent respectively. Immediate reactions were obtained with the first of the 2 solutions of the glucoside, but with the second, that containing .00015 per cent of nitrogen, only questionable immediate reactions occurred. The reactions with the .00036 per cent solution were peculiar in that only 4 of the 12 strongly sensitive patients showed positive tests. Possibly the glucoside was associated with a particular nitrogenous substance to which all patients were not sensitive. Control tests were negative.

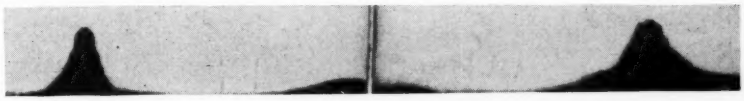

Fig. 1.—Electrophoretic pattern of 0.1 per cent solution of isoquercitrin in borate – NaOH–HCl buffer at pH 8.4 at the end of a 60 minute run. Mobility in ascending limb (left pattern), 11.9 cm. $\times 10^{-5}$.

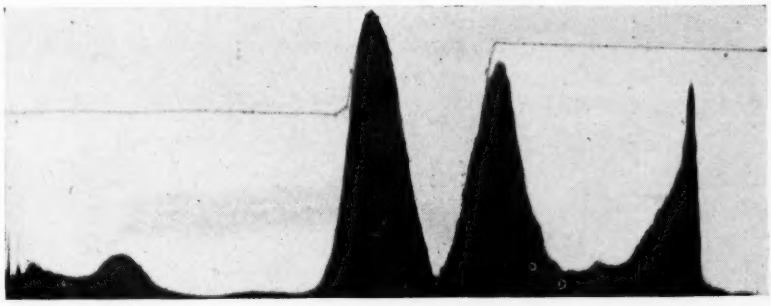

Fig. 2.—Undialyzed giant ragweed extract with added buffer (borate – NaOH-HCl), at pH 8.4 after 60 minutes. Mobility of fast pigment in ascending limb, 11.6 cm. $\times 10^{-5}$.

Electrophoretic Patterns.—Difficulties were encountered in obtaining the electrophoretic pattern of whole ragweed extracts because in dialysing to bring the extracts to the desired pH and into equilibrium with the buffer solutions used electrophoretically, all the components in the pattern were found dialysable, but by the gradual addition of a concentrated alkaline buffer solution the extracts could be brought to the desired pH, thus avoiding dialysis. In comparing whole ragweed extracts with the solutions of isoquercitrin, a pH more alkaline than that usually employed for electrophoresis was required because of the insolubility of the isolated isoquercitrin. A pH of 8.2 to 8.4 was most satisfactory. Concentrated buffer solutions (borate – NaOH-HCl [pH 8.4]) and concentrated solutions of ragweed were prepared and buffer

was added to the ragweed solutions until the desired pH was attained. The ragweed solutions were then diluted with distilled water to contain 50 mg. of nitrogen per 100 c.c. The buffering solutions employed electrophorectically were made by diluting the solutions of concentrated buffer in the same proportion with distilled water as they were diluted in the ragweed extracts. Isoquercitrin was dissolved in this diluted buffer to make a 0.1 per cent solution. These solutions were satisfactory except for some salt interference in the patterns.

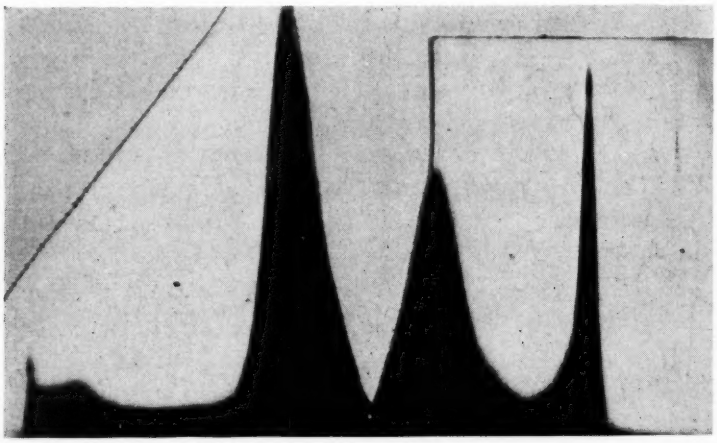

Fig. 3.—Pattern of a concentrated dialyzate containing 50 mg. N per 100 cc., buffered at pH 8.4 with borate – NaOH-HCl, after 61 minutes. Mobility of fast pigment in ascending limb, 11.9 cm $\times 10^{-5}$.

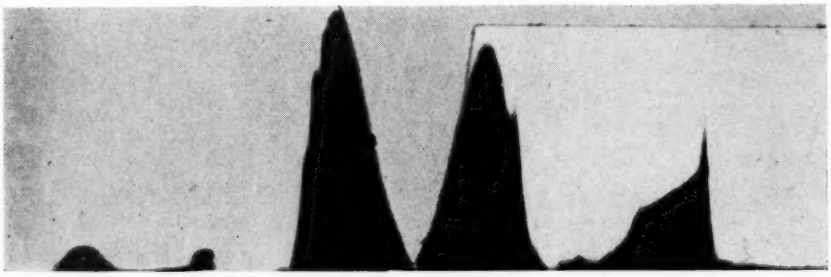

Fig. 4.—Giant ragweed extract with two-thirds of isoquercitrin precipitated. Borate – NaOH-HCl buffer at pH 8.4, 60 minute run. Mobility of fast pigment in ascending limb, 11.9 cm. $\times 10^{-5}$; 50 mg. N per 100 c.c.

The mobility of the isoquercitrin was found to be 11.9 cm. $\times$ 10^{-5} (pH 8.4) in both the ascending and descending limbs of the U tube. This mobility was compared with that of the rapidly moving pigment in solutions of ragweed extracts prepared in several ways. All the ragweed extracts studied contained 50 mg. of nitrogen per 100 c.c. In the first experiment whole ragweed extract was diluted with borate buffer but not dialyzed, while in the second the extract was dialyzed against the borate buffer solution for 24 hours. In the third experiment, extract was used which had been refrigerated for 4 weeks with partial precipitation of the isoquercitrin. In the final experiment

an extract was concentrated by dialyzing under pressure, washed with several volumes of distilled water, and then diluted with borate solution to bring it to the desired pH and concentration of nitrogen. In all 4 experiments the main component in the patterns was clearly delineated but showed fragmentation. At pH 8.4 the mobility of this slowest component was 0.6. In the ascending limb in the first 3 experiments the mobilities of the fast moving pigment were 11.6, 11.9, and 11.9, respectively.[2] In the fourth experiment this pigment component was absent, apparently having been washed out completely by the dialysis. Because of the identical mobilities of isoquercitrin (11.9 at pH 8.4) and of the fast pigment, this flavone glucoside is believed to be one of the constituents of this pigmented component.

Fig. 5.—Giant ragweed extract dialyzed and concentrated under pressure; rapidly moving pigment dialyzed out, 120 minutes; 45 mg. N per 100 c.c.

SUMMARY

In the preceding experiments isoquercitrin, a tetrahydroxy-flavone glucoside, has been isolated from giant ragweed pollen and found to be a constituent of the most rapidly moving pigment in the electrophoretic pattern of the pollen. The crystalline pigment isolated from the ragweed extracts was inactive biologically on cutaneous testing, a result anticipated because this pigment is so widely distributed in various plants that a specific role in the activity of the pollen could not be expected. But the freshly isolated pigment in amorphous form, before drastic procedures were undertaken to cause its crystallization, contained nitrogenous material with specific biologic action in the skin of ragweed-sensitive patients. When the nitrogen content of the amorphous pigment was reduced fourfold positive cutaneous tests were no longer obtained, presumably indicating that the activity was inherent in the nitrogenous fraction or fractions associated with the pigment. The nature of this association, whether a chemical combination or merely a chemical contamination, is not clear, but numerous unpublished experiments suggest that the active nitrogenous fraction is combined with the pigment. Since the mobilities of the isolated isoquercitrin and the pigment in the whole ragweed solution are identical such a combination would necessarily be through linkage with the glucose of the glucoside.

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STUDIES IN CONTACT-DERMATITIS*

VIII. The Effect of Feeding of Antigen on the Subsequent Development of Skin Sensitization

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IN EXPERIMENTS on the guinea pig, Chase[1] demonstrated that feeding of certain chemicals to the nonsensitive animal established a blocking effect of substantial degree against subsequent sensitization of the skin by the same agent. A 1 per cent solution of dinitrochlorobenzene in olive oil in amounts of 0.3 ml. was fed the animals from the tip of a pipette. The feedings were made daily for 6 days, and were followed by a rest period of 8 days. After 2 or 3 such courses and a rest period of 2 weeks, the animals were given 6 or more intracutaneous injections of the antigen over a period of several weeks. Two weeks after the final injection a contact test was performed. A blocking effect was demonstrated. Though it was not absolute, it was of significant magnitude in a large number of animals, it was specific, and it was durable (up to 31 weeks).

This work naturally raised the question of the role of the ingestion of antigen as an inhibitor of skin sensitization in human beings. In re-examinating notes on unpublished experiments in 1937 on sensitization of the human skin with krameria[2, 3] I found that a group of 9 subjects had been given krameria orally prior to an attempt to induce sensitivity of the skin to the same excitant. This particular experiment had been carried out to determine whether the ingestion of krameria would influence the development of skin sensitization. Since complete inhibition had not been demonstrated, and since the majority of the subjects had become sensitized at some stage in the investigation, the study was discontinued.

After Chase's reports on animals, my findings were re-analyzed. Though the data suggested that some inhibitory effect had been produced by the prior ingestion of the antigen, the evidence could not be evaluated fully since planned controls had not been included. Therefore the investigation was resumed recently under more favorable experimental conditions. The findings in this study are presented in this report.

The subjects employed were adults, either patients in the allergy clinic under treatment for hay fever, allergic rhinitis, and asthma, or mothers of allergic children. While it was originally intended to include at least 20 test subjects and a like number of controls, the final number who cooperated up to the completion of the experiment was smaller.

There were 11 test subjects, 1 male and 10 females, ranging in age from 17 to 54 years. Twenty-four ounces (710 ml.) of the official tincture of krameria (U.S.P.X) was established as the total dose. It was to be taken over a period of 8 weeks. Each subject was instructed to drink 1 teaspoonful (approximately 4 ml.) of the medication diluted in 1 ounce (30 ml.) of water 15 minutes before meals. One-half of the subjects were to continue this procedure for the full

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dose. In the other subjects there were alternate periods of ingestion of the excitant followed by rest periods as follows: 1 teaspoonful (approximately 4 ml.) of the drug was to be taken 3 times daily for 2 weeks, then after a rest period of 1 week, 2 teaspoonfuls (approximately 8 ml.) of the medication were to be taken for 1 week, followed by another rest period of 1 week. This schedule was followed until the full dose had been taken.

The alternate plans had been prescribed in order to determine any difference in protective effect in respective groups. Actually it was possible to persuade only 4 of the subjects to take the drug continuously, and 7 took it in interrupted courses. In a few instances there were slight variations from the established procedures, but the general plan was carried out for the most part.

The procedure of sensitization was begun 2 weeks after the last dose of the drug had been taken. The fluidextract of krameria (N.F. VII) was used as the antigen. This preparation represented 1 Gm. of the dried drug in 1 ml. of the vehicle which contained 60 per cent alcohol. The tincture employed in the feeding experiments contained an amount of drug which compared to that in the fluidextract in the ratio of 1:5.

To induce sensitization a one-half inch square of white blotting paper was saturated with 3 drops of the excitant and applied as a patch test to the unbroken skin of the arm for 24 hours. If no reaction ensued the application was repeated at an adjacent area for the same period. In the absence of a reaction, this procedure was repeated each week. In a few instances there were unavoidable minor variations from this plan (see Table I).

There were 10 control subjects, all women, ranging from 27 to 56 years of age. Sensitization was induced in a manner identical with that employed in the treated subjects. There was an occasional unavoidable deviation from the general plan (see Table II). It was not believed that such minor deviations could influence the final outcome.

RESULT

Test Subjects.—Sensitivity was induced following from 1 to 4 applications of the excitant (Tables I and II). It occurred after 4 patch tests in 3 subjects, after 3 in 2 subjects, after 2 in 4 subjects, and after a single application in 2.

The intensity of reactions was graded in 3 degrees: a mild response consisted of erythema and infiltration; a moderate, of papule and early vesicle formation; and a marked, of distinct vesiculation. There were 4 mild, 5 moderate, and 2 marked reactions. As another index of intensity of reaction, spontaneous flare-up[3] occurred in 4 subjects (Table I).

Control Subjects.—Sensitivity was induced in the controls after from 1 to 5 applications of the antigen (Tables II and III). It occurred after 5 patch tests in 1 subject, after 4 in 3 subjects, after 3 in 2 subjects, after 2 in 3 subjects, and after a single application in 1. There were 3 mild, 4 moderate, and 3 marked reactions. Spontaneous flare-up occurred in 5 subjects.

DISCUSSION

I realize that a series of 11 test and 10 control subjects is a small one indeed. It was difficult, however, to get thorough cooperation in a large group of human

TABLE I.

SUBJECT	SEX	AGE	FEEDING METHOD*	DIAGNOSIS	APPLICATION 1		INTERVAL IN WEEKS BETWEEN APPL. 1 & 2
					DURATION IN DAYS	REACTIN	
S. B.	F	44	I	All. rhinitis polyps	1	0	2
J. F.	F	18	C	All. rhinitis	1	0	2
G. M.	F	19	C	All. rhinitis	2	0	2
I. F.	F	37	I	All. rhinitis	1	0	2
Y. K.	F	49	I	Br. asthma	1	0	2
R. G.	F	33	I	All. rhinitis	1	0	2
L. G.	F	17	I	Br. asthma	1	0	2
M. S.	F	49	I	Hay fever	1	0	2
A. C.	F	54	C	All. rhinitis	1	0	3
S. S.	M	39	I	Br. asthma	1	mod.	
A. G.	F	44	C	All. rhinitis Br. asthma	1	mod.	

*I = Interrupted.
C = Continuous.

TABLE II.

SUBJECT	SEX	AGE	DIAGNOSIS	APPLICATION 1		INTERVAL IN WEEKS BETWEEN APPL. 1 & 2	APPLICATION 2		INTERVAL IN WEEKS BETWEEN APPL. 2 & 3
				DURATION IN DAYS	REACTION		DURATION IN DAYS	REACTION	
J. M.	F	32	*	1	0	2	1	0	1
S. E.	F	38	*	1	0	2	1	0	2
M. W.	F	39	*	1	0	2	1	0	1
A. T.	F	38	Hay fever	1	0	2	1	0	1
E. D.	F	27	*	1	0	2	1	0	1
M. WE.	F	46	Hay fever	2	0	2	1	0	1
M. Z.	F	27	*	1	0	2	1	mod.	
B. Z.	F	27	*	1	0	2	1	mod.	
A. M.	F	28	*	2	0	2	1	mkd.	
F. R.	F	56	Hay fever	2	mild				

*Mothers of allergic children.

subjects because of the unpleasant taste of the drug and the discomfort brought on by the positive reactions on the skin. There were some who started the experiment but did not continue it to completion.

Careful examination of the results in this series, however, leads one to the conclusion that in terms of intensity of reaction and number of patch-test applications required to produce sensitivity there was no essential difference in the test and control groups.

Chase[1] observed that the protection afforded by prior feeding of antigen was not absolute; it remained possible that the protective effect could be overridden by intensive treatment with the allergen. In my study in 1937 on human subjects, neither the feeding of antigen nor patch-test treatment with it followed any established procedure. One got the impression, however, that some inhibitory effect might be present, but it was not definite because of lack of suitable controls. From the present series, no such difference in the end result could be demonstrated.

It is to be noted, moreover, that the feeding of antigen was stopped 2 weeks prior to the first sensitizing application of the excitant. This procedure had been adopted in order to parallel the general plan followed by Chase.[1] Whether

TEST SUBJECTS

APPLICATION 2		INTERVAL IN WEEKS BETWEEN APPL. 2 & 3	APPLICATION 3		INTERVAL IN WEEKS BETWEEN APPL. 3 & 4	APPLICATION 4		SPONTANEOUS FLARE-UP AT SITE
DURATION IN DAYS	REACTION		DURATION IN DAYS	REACTION		DURATION IN DAYS	REACTION	
1	0	1	1	0	1	1	mod.	2–3
1	0	1	1	0	1	1	mod.	3
1	0	1	1	0	1	1	mild	
1	0	1	1	mod.				2
1	0	2	1	mkd.				1–2
1	0	1	1	mild				
1	mild							
1	mod.							
1	mkd.							

CONTROL SUBJECTS

APPLICATION 3		INTERVAL IN WEEKS BETWEEN APPL. 3 & 4	APPLICATION 4		INTERVAL IN WEEKS BETWEEN APPL. 4 & 5	APPLICATION 5		SPONTANEOUS FLARE-UP AT SITES
DURATION IN DAYS	REACTION		DURATION IN DAYS	REACTION		DURATION IN DAYS	REACTION	
1	0	1	1	0	3	1	mod.	4
1	0	1	1	mild				
1	0	1	1	mild				
1	0	1	1	mod.				3
1	mkd.							1—2
1	mkd.							1—2
								1

TABLE III. REACTIONS OF SENSITIZATION

	NUMBER OF TEST SUBJECTS	NUMBER OF CONTROL SUBJECTS
Number applications required to sensitize		
1	2	1
2	4	3
3	2	2
4	3	3
5		1
Intensity of reactions		
mild	4	3
moderate	5	4
marked	2	3
Total subjects	11	10

the continued ingestion of antigen would have altered the final outcome can be determined only by further study.

SUMMARY

Eleven adults ingested 24 ounces (710 ml.) of the tincture of krameria in teaspoonful doses (approximately 4 ml.) over a period of 8 weeks. Two weeks

later patch-test applications were made with the fluidextract of krameria. In the absence of any reaction they were repeated at spaced intervals until evidence of sensitization was demonstrated. There were 10 control subjects in whom repeated patch-test applications were made according to the procedure followed in the prepared subjects.

All test subjects and controls were sensitized. No difference existed in both groups, either in intensity of induced reactions or in the number of patch-test treatments required to provoke sensitization.

CONCLUSION

The feeding to human beings of an antigen, krameria, did not produce a blocking or inhibiting effect to subsequent skin sensitization with the same antigen.

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POLLEN TANNATE SUSPENDED IN PEANUT OIL WITH ALUMINUM MONOSTEARATE IN THE TREATMENT OF HAY FEVER*

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TANNIC acid precipitates[1, 2] of the active fractions of pollen extract have been used for the desensitization treatment of pollinosis since 1939, except for the years 1942 to 1945. It was shown[1] that the active principle in pollen extract solutions was precipitated by the tannic acid as indicated by inability of the supernatent to produce positive skin reactions in sensitive patients, and by the production of local and general reactions in sensitive patients injected with tannate precipitates. Desensitization after treatment with tannate precipitates was proved by reduction in skin sensitivity, the ability to tolerate large doses of regular extract, and the satisfactory clinical results. In a preliminary study in 1949, 28 patients treated with grass and ragweed precipitates suspended in peanut oil with aluminum monostearate had encouraging results. The purpose of this paper is to describe the present method of preparation of pollen tannate precipitates, and to report results of treatment in 1950 with such precipitates suspended in peanut oil with 2 per cent aluminum monostearate.

METHODS

Preparation of Materials.—Short ragweed pollen was used. The grass preparation was made from equal parts of timothy and orchard grass pollen. Pollen is extracted with ether, dried, and then extracted with physiologic saline solution to make a 1:20 solution. The extract is filtered and measured. A solution of 10 per cent zinc sulfate is added sufficient to make a 0.1 per cent concentration. Then a freshly prepared 10 per cent tannic acid solution is added to make a 0.5 per cent final concentration. The resulting flocculation is allowed to settle. The supernatant is removed. The remainder is centrifuged lightly, the supernatant removed, and the sediment of pollen tannate is rubbed into a small volume of 95 per cent ethyl alcohol to break up any clumps. At this point the sediment becomes dark brown. To remove excess tannic acid, water, and other nonprotein pollen fractions, the sediment is extracted repeatedly with alcohol until the supernatant is clear and colorless. The sediment is finally taken up in sufficient 95 per cent alcohol containing 2 per cent phenol to make a 1:1 relation, that is, if 100 Gm. of pollen were originally extracted and 1,800 c.c. of 1:20 dilution obtained, the final volume is 90 c.c. The suspension is placed in a sterile bottle, capped, and after being tested for sterility, is ready for use and stored as stock.

This method may be modified readily. If the dry pollen is extracted fractionally with small portions of saline solution, or by continuous saline drip, the active material will be practically completely removed from the pollen. The final suspension may then be considered to contain all the active material from the original weight of pollen and the volume adjusted accordingly, that is if

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100 Gm. of pollen were used originally, the final volume of 1:1 extract is 100 c.c. Although desirable, it is difficult to carry out these procedures with sterile technique, especially when dealing with large quantities. For practical purposes, sterile technique is not necessary since the use of 2 per cent phenol in 95 per cent alcohol to make the final suspension ensures chemical sterilization.

If dilutions of the suspension in saline solution are to be used for treatment, it is advisable to make the final 1:1 stock suspension in glycerin instead of 95 per cent alcohol, since a 1:10 dilution in saline solution of the alcoholic stock suspension causes pain on subcutaneous injection.

To make a suspension in oil with aluminum monostearate, using sterile technique, the desired amount of the alcoholic stock suspension is centrifuged lightly, and the supernatant removed. The sediment is taken up in ether to the original amount, centrifuged again, the supernatant removed, and the sediment is rubbed and stirred into a small volume of the oil with aluminum monostearate, making certain a homogeneous suspension is present before adding oil sufficient to making a 1:1 suspension. Dilutions are made by adding the 1:1 suspension in oil to sterile peanut oil with 2 per cent aluminum monostearate.

Standardization of Preparation.—Attempts were made to standardize the stock suspension 1:1 in alcohol by nitrogen determination of the whole suspension. The only method available was the micro-Kjeldahl, which gave very variable results. The regular Kjeldahl method would probably give more accurate, consistent results. Because of the variable nitrogen determinations on the same preparation and the use of different lots of suspensions during the course of the study, the weight-volume standardization is used here. However, it may be stated that the average content of tannic acid-precipitable N_2 was about 1.5 mg. per cubic centimeter of the 1:1 stock. It is generally estimated that 1 c.c. of 1:1 pollen extract (1.0 Gm. of pollen) should yield about 5.0 mg. of protein N_2 by phosphotungstic acid precipitation.[3] However, the tannic acid precipitation was about one-third the estimated yield. This discrepancy requires further study.

Method of Treatment.—Injections were given subcutaneously in the arms every 7 to 14 days using a tuberculin syringe with an ordinary hypodermic needle (26 gauge, one-half inch). The dilutions in oil used in treatment were 1:1, 1:10, 1:100, and 1:1,000. Patients who were considered to be very sensitive by skin test, especially patients with no previous treatment, were started with 0.1 c.c. of the 1:1,000 dilution. Patients who had lesser skin test sensitivity or had had previous treatment were started with the 1:100 dilution, occasionally with the 1:10 dilution. Usually, subsequent doses were increased by 3 times, thus: Dose 1, 1:1,000, 0.1 c.c.; Dose 2, 1:1,000, 0.3 c.c.; Dose 3, 1:100, 0.1 c.c., and so on. Often a fivefold or tenfold increase in dosage was given if a very small reaction occurred from the previous injection. The maximum dose arbitrarily selected was 0.1 c.c. to 0.3 c.c. of the 1:1 suspension. If an excessive reaction occurred with a particular dose, the same dose was repeated and practically always a small reaction resulted. When the maximum dose was reached, it was repeated once if necessary to make sure the reaction was slight to mod-

erate, and then was repeated every 4 to 6 weeks until the beginning of the pollen season. Usually no injections were given during the season.

A patient sensitive to grass and ragweed pollen was treated with a mixture of equal parts of grass and ragweed suspension. After June 1, no further grass pollen was given, and desensitization was continued with ragweed alone until the early part of August. The tree preparation consisted of equal parts of 1:1 birch and 1:1 oak pollen tannate in oil.

Selection of Patients.—Only patients with pollen sensitivity of moderate or severe degree by both history and skin test were selected for treatment. The degree of skin sensitivity was determined by measuring the wheal and erythema produced by scratch tests using solutions of pollen extract in 50 per cent glycerin in dilutions of 1:50, 1:500, 1:5,000. These tests were repeated in most cases during and at the end of the course of treatment to detect desensitization, objectively.

The patients' ages ranged from 5 years to 83 years. Some were treated for 2 to 3 different pollen seasons. The number of pollen seasons treated is used as the number of patients for convenience in presenting results. The maximum dose usually was reached early and was repeated at intervals of 4 to 6 weeks until the onset of the season. The total number of injections is therefore affected by the date when the patients started treatment; the number of injections required to reach the maximum dose is not so affected. For this reason, the number of injections required to reach the maximum dose indicates the efficiency of the method. The number of injections needed to reach a certain dose in any one patient may be reduced because of persistent desensitization from previous treatment; therefore the grass and ragweed patients who had no previous treatment are presented separately.

RESULTS

An analysis of the method of treatment considers (1) the starting dose, (2) the maximum dosage, (3) the number of injections required to reach the maximum dose, and the total number of injections, and (4) the clinical results divided into the local and general reactions, and the symptomatic relief.

The Starting Dose.—The starting doses for each pollen season are presented in Table I. The dose was usually 0.1 c.c. of the particular dilution; occasionally 0.05 c.c. or 0.15 was given. The 1:1,000 dilution was usually used for new patients or patients with very large skin reactions. In general, the starting dose was about 10 times the usual starting dose when regular pollen extract is used.

The Maximum Dose.—The maximum dose ranged from 0.1 c.c. to 0.3 c.c. of the 1:1 dilution. When the concentrated 1:1 preparation is used, small differences in volume represent very large differences in units. Most, if not all, patients who were maintained on a dose of 0.1 c.c. of 1:1 dilution could have been raised higher. The smaller doses were used to observe the results with different dosage, and to conserve material. The 0.1 c.c. dose, theoretically 100,000 noon pollen units, was estimated to contain at least 10,000 to 20,000 protein nitrogen units.

Number of Injections.—The number of injections required to reach the maximum dose is very small, averaging from 4.3 to 5.8 (Table I). The patients who had had treatment in previous years, usually with pollen tannate suspended in water, had some desensitization remaining, and their degree of sensitivity was known from experience. Therefore they were usually started with a higher dose, and frequently their increase in dosage was greater. The previously untreated patients present no such factors influencing treatment, and their figures are given in Table II. The average number of injections required for the new patients was 6.3 to 7.4. To reach a similar maximum dose of regular extract would require ordinarily 3 or more times that number of injections.

TABLE I. 1950 TREATMENT WITH POLLEN TANNATE SUSPENSION IN OIL WITH 2 PER CENT ALUMINUM MONOSTEARATE

	NUMBER OF PATIENTS	STARTING DOSE DILUTION			AVERAGE NUMBER OF INJECTIONS TO MAXIMUM DOSE	AVERAGE TOTAL INJECTIONS
		1:1000	1:100	1:10		
Trees	9	0	6	3	4.3	5.5
Grass	31	4	23	4	5.7	7.6
Ragweed	82	18	49	15	5.8	9.1

The total number of injections for each pollen season are given in Tables I and II, and ranged from 5.5 to 10.6. This factor is influenced by the time of starting treatment; the earlier treatment was started, the longer the period for repeating the maximum dose. It is probable that desensitization was increased by repeated maximum doses.

TABLE II. PATIENTS WITH NO PREVIOUS HAY FEVER TREATMENT

	NUMBER OF PATIENTS	AVERAGE NUMBER OF INJECTIONS TO MAXIMUM DOSE	AVERAGE TOTAL INJECTIONS
Grass in oil	8	6.3	7.9
Ragweed in oil	30	7.4	10.6

Local and General Reactions.—The peanut oil with 2 per cent aluminum monostearate in the amounts used had no gross tissue reaction. Whenever a local reaction occurred, it seemed related to the pollen tannate. The local reactions in general were similar to the local reactions produced by regular extracts. They developed much more slowly, however, with the greatest reaction occurring 1 or 2 days after injection. The slight to moderate reactions disappeared in 1 to 4 or 5 days, with no residual. The larger reactions showed diffuse swelling for several days, and frequently showed a small induration, which gradually disappeared, at the area of injection for 7 days or more thereafter. Occasionally a small, barely palpable, fibrotic nodule remained at the site of the more intense local reactions for several weeks. Usually even very large local reactions were not accompanied by general reactions. General reactions occurred very seldom, started 1 to 3 hours after injection, developed gradually, were mild enough to be treated by the patient with an antihistamine or ephedrine tablet, or required no treatment.

Two patients developed sterile abscesses at the site of tree pollen in oil injections. One, a ragweed-sensitive patient, received by error an injection of 0.1 c.c. of 1:1 tree pollen tannate in oil. He developed a walnut-sized induration which became fluctuant and healed without incident after incision and drainage done 6 weeks after the injection. The second patient, who had severe chronic atopic dermatitis, was sensitive to tree, grass, and ragweed. In the arm which received tree tannate in oil, she developed 3 nodules which became fluctuant and required incision and drainage 3 months after the last tree pollen injection. No complications appeared in the arm which received the grass and ragweed preparations. The tendency to induration was much greater with the tree preparations. It is likely there was some nonspecific irritant extracted from the tree pollen which accounted for this. The induration in the patient not sensitive to trees suggests this. About 1,000 grass and ragweed injections showed no such complications.

Skin Tests.—Objective evidence of desensitization is the effect of treatment on the degree of sensitivity measured by the skin test. Practically all patients had scratch skin test measurements done before treatment and most were repeated at the end of treatment. In general, most patients showed definite decrease in skin test sensitivity, and in many cases the tests became practically negative.

Symptomatic Relief.—There is no objective method of evaluating symptomatic relief. The patients were questioned about their symptoms and medication at intervals during and at the end of the season. They were classified as having satisfactory symptomatic relief, or not. In 6 instances the results were equivocal and could not be placed in one or the other category. The satisfactory group ranged from definite improvement with the help of occasional small doses of antihistamines to practically complete absence of symptoms without any medication. The results according to size of maximum dose given are not presented since it is not possible at this time to state with certainty the variations in results. Eleven patients did not report after their season had started, and results in their cases could not be evaluated. The results in the others are presented in Table III; 90.0 per cent of the cases had satisfactory results, 5.4 per cent were equivocal, and 4.5 per cent had unsatisfactory results. It is our impression that even the patients with unsatisfactory results had some degree of relief, and would have been worse without treatment. They showed evidence of desensitization by skin test, and tolerated their doses well. Factors other than the specific pollen sensitivity probably influence the symptoms of

TABLE III. SYMPTOMATIC RELIEF

	NUMBER OF PATIENTS	UNSATISFACTORY	EQUIVOCAL	SATISFACTORY
Ragweed	74	4	4	66
Grass	29	1	2	26
Trees	8	0	0	8
Total	111	5 (4.5%)	6 (5.4%)	100 (90.0%)

patients[4] and it is unlikely that specific desensitization can completely relieve all patients. The results obtained compare favorably with regular methods of treatment.

DISCUSSION

Antihistaminic drugs are valuable in the treatment of pollinosis. Adrenal cortical hormones may prove effective and practical. However, desensitization by injections continues to be a necessary procedure in most patients with more than slight sensitivity. Since the introduction of desensitization therapy by Noon 40 years ago, no significant change in the preparation or administration of pollen extracts has occurred. In general, the greater the desensitization, the greater the relief of symptoms, and the greater the chance for persistant desensitization approximating a "clinical cure." Attempts to obtain more effective desensitization have been concerned mostly with methods of delaying and prolonging absorption of the pollen extract. One effective method is the emulsification of solutions of pollen extract in oil using as the emulsifying agent cholesterol substances such as lanolin, Aquaphor, or Eucerite.[5, 6] Another method converts the water-soluble pollen extract to an insoluble precipitate by means of tannic acid and zinc,[1, 2] or hydrochloric acid.[7] The preparation used in this report combines the important features of both these methods with greatly increased effectiveness. The same principles applied to penicillin very successfully prolong absorption and the effect of each injection. The very large quantities of pollen extract tolerated by these means and the comparatively few injections needed to accomplish significant desensitization are striking.

It is likely that increasing the viscosity of the preparations by adding cholesterol agents such as lanolin, Aquaphor, and Eucerite, or making solid pellets for implantation, will increase the effectiveness still further, so that 1 or 2 injections may suffice for desensitization. Suspensions of pollen tannate in gelatin[8] may be effective and should be tried. There is some indication that treatment of pollen tannate precipitates with formalin reduces the capacity to produce immediate reactions, but may nevertheless desensitize. Dry lyophilized pollen extract suspended in oil[9] probably cannot be as effective as the method presented since the suspended material is water soluble, whereas pollen tannate is not.

Another advantage in using the oil suspension concerns the factor of safety. The dreaded severe acute general reaction due to the unpredictable rapid absorption of water solutions of pollen extract is much less likely to occur with oil suspensions. Many patients cannot exceed a certain dose of solutions without developing general reactions, and often this tolerated dose may not desensitize effectively. No such instances have been encountered with the use of oil suspensions. A patient treated with solutions who skips a weekly visit usually must have the last dose repeated; with oil suspensions the next scheduled increase may be given safely, and thus time is saved. With the use of large doses, a greater degree of desensitization results as indicated by the reduction in size of skin tests and by the smaller number of injections needed to reach the same

or larger dosage in successive years of treatment. The possibility of a clinical "cure" is thus greatly increased by a method which permits the administration of larger dosage.

Several patients had coseasonal treatment with oil suspensions given twice a week. Although in general such patients claimed improvement, the number is too small to warrant drawing any conclusions. No perennial treatment was given since the number of preseasonal injections was smaller than the usual number of perennial injections. However, it may be possible to give perennial treatment with oil suspensions at intervals of 8 weeks, or longer, in which case perennial treatment may be more desirable.

A major problem with solutions of pollen extract concerns the deterioration of activity which makes it unwise to prepare large quantities of material, makes it difficult to change treatment from an old preparation to a fresh one, and necessitates refrigeration of extracts. The factors, possibly enzymes, responsible for the deterioration apparently are eliminated in the process of precipitation. The precipitates, in a dehydrated state in alcohol, and the oil suspensions used in treatment were stored at room temperature. In large scale commercial production, the storing of the precipitate in the dry powdered state might be more practical.

House dust precipitates have been made and used, but not sufficiently to warrant a report. The methods described should be applicable to any pollen, mold, and other antigens.

A patent on the use of tannic acid to make pollen precipitates has been issued and assigned to Eli Lilly and Company.[10] Since the patent application followed the publication of the original paper, the right to sublicense under the patent has been secured by the author, who will grant sublicenses freely.

SUMMARY AND CONCLUSIONS

1. Water suspensions of the active fractions of pollen extract precipitated by tannic acid and zinc have been used effectively for specific pollen desensitization since 1939. Suspensions of pollen tannate in peanut oil with 2 per cent aluminum monostearate were used in 1949 and 1950.

2. The methods of preparing such suspensions are described.

3. The advantages of using pollen tannate suspensions in peanut oil with 2 per cent aluminum monostearate depend on slowing and prolonging the absorption of the active material resulting in (a) increased safety and tolerance of larger doses, and (b) increased stability of the active material due to its dehydrated state, and possible elimination of enzymes.

4. The results of treatments, in 1950, of 122 patients with pollinosis with pollen tannate suspended in peanut oil with 2 per cent alminum monostearate are presented. A final dose of 0.1 c.c. to 0.3 c.c. of a 1:1 suspension in oil, representing about 15,000 to 45,000 protein nitrogen units, was attained by an average number of 4.3 to 7.4 injections, with 90.0 per cent satisfactory results.

5. This new method of preparing and administering desensitizing antigens presents opportunities for further experimentation.

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VITAMIN B_{12} THERAPY IN ALLERGY AND CHRONIC DERMATOSES

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INTEREST in the use of vitamin B_{12} in asthma was first aroused by the work of Wetzel, Fargo, Smith, and Helikson on the response of malnourished children to this vitamin.[1] The case cited was of a boy with severe allergic bronchitis whose sleep for 12 months previously had been regularly interrupted by asthmatic attacks and whose daytime wheezing permitted little desire for food, not to mention time for eating it. His response in respect to growth was accompanied by a remarkable attentuation of symptoms; in fact, these symptoms vanished during the first week.

In order to evaluate therapy with B_{12} in asthma, 20 adult patients were selected. Nineteen of these had, heretofore, intractable asthma; the other patient had mild asthma but severe eczematoid atopic dermatitis. All patients were either under weight or of normal weight. In order to make certain that adequate amounts of B_{12} were actually absorbed, 1,000 μg* were injected intramuscularly at weekly intervals until 4 injections had been given. All patients had complete blood counts, weights, and vital capacity determinations before starting treatment, 1 week after the final injection, and after another 3 weeks. If specific allergic or vaccine treatment was being given, this was continued, as was oral or aerosol medication. A distinct effort was made to continue the treatment used prior to B_{12} administration throughout the 7 week period required for the study.

The ages of the patients were from 30 to 75 years with an average of 55.6 years. Objective findings after B_{12} were as follows: 12 patients gained weight, 6 lost, and 2 remained the same. The gain was from 1 to 6 pounds and averaged 3 pounds. The loss was from 0.5 to 4.5 pounds, the average being 2.5 pounds. The average weight change was a gain of a little over one pound per patient. Vital capacity, which in all but one patient was originally below normal, showed an increase in 14, a decrease in 2, and was unchanged in 4, or an average increase of +9 per cent per patient. The maximum increase was +44 per cent and the maximum decrease was −10 per cent. Red blood counts, hemoglobin, white blood counts, and differentials showed no definite pattern and variations were within experimental error. Subjectively, 18 of the patients stated that there was a definite improvement in reference either to asthma, shortness of breath upon exertion, appetite, sleep, or general condition (Table I). This improvement did not always agree with an increased vital capacity, was not spectacular, and could have been due in some measure to the institution of new treatment. In no patient was the improvement sufficient to consider discontinuance of other therapy. This would have completed our investigation except

*Furnished through the courtesy of Merck and Co., Inc., Rahway, N. J.

that the one patient who had both asthma and atopic dermatitis had a very definite and spectacular clearing of his chronic dermatitis. Therefore, further investigations were carried out on chronic dermatoses.

TABLE I. VITAMIN B_{12}; EFFECT ON 20 ASTHMA PATIENTS

Weight	12 gained (av. 3 lb.)	6 lost (av. 2.5 lb.)	2 same
Vital capacity	14 increased	2 decreased	4 same
Subjective results	18 better	2 same	

Twenty-eight patients with chronic dermatoses were given B_{12} in the same manner and dosage as was done with the asthmatic patients. Again there was little if any change in the blood or differential counts. The difference in weight (gain or loss) apparently had nothing whatever to do with the amount of relief obtained. Ten patients were diagnosed as having atopic dermatitis; 1 showed great improvement, 5 moderate, and the other 4 none. Six patients with chronic contact dermatitis, all of whom were unimproved with local treatment and elimination of the causative agent, started clearing with the institution of B_{12} therapy and were greatly improved or entirely clear at its conclusion. Two patients with dermatitis herpetiformis remained unimproved. Ten patients with chronic urticaria, in all of whom the condition had existed for more than one year, were treated; 9 were greatly improved or cured and one was definitely worse. This latter patient was found to have an intestinal infection and with the institution of Chloromycetin therapy the urticaria cleared (Table II).

TABLE II. VITAMIN B_{12}; EFFECT ON CHRONIC DERMATOSES

CONDITION	NUMBER OF PATIENTS	RESULTS	
		NUMBER	EXTENT IMPROVED
Atopic dermatitis	10	1	Greatly
		5	Moderately
Chronic contact dermatitis	6	6	Greatly improved or cured
Dermatitis herpetiformis	2	0	
Chronic urticaria	10	9	Greatly

DISCUSSION

The distinction between atopic and contact dermatitis was made by these criteria: In contact dermatitis there was in most cases no history of personal or familial atopic diseases. The lesions were usually acute or subacute moderately pruritic dermatoses consisting of one or more of the following: erythema, edema, vesicles, bullae, papules, scaling, infiltration, and lichenification the latter generally involving exposed areas. Patch tests were done on all patients and at least one was positive on each patient. In atopic dermatitis there was generally a history of personal or familial atopic diseases. The lesions were usually papular, lichenified, and highly pruritic, with predilection for cubital and popliteal spaces, eyelids, sides of neck, and dorsa of hands and fingers. Intradermal tests were done on all patients and some positive whealing reactions resulted.[2]

The response in chronic urticaria would lead one to believe that more could be expected in atopic dermatitis which "is a combination of subclinical urticaria with eczematoid characteristics."[2] However, such was not the case.

Where there was a definite relationship between the atopic dermatitis and worry, fear, or anxiety of the patient ("neurodermatitis"), no result was obtained with B_{12} therapy.

CONCLUSIONS

No absolute conclusions can be drawn from the small number of patients treated in each category. The only conclusions that can be reached after a perusal of the cases are the following:

1. Vitamin B_{12} is probably of no value in the treatment of chronic bronchial asthma.
2. Vitamin B_{12} is probably valueless in the treatment of chronic dermatoses except that when other therapy fails in chronic contact dermatitis and in chronic urticaria it may possibly be worth a trial.

SUMMARY

Vitamin B_{12} therapy using 1,000 μg. doses intramuscularly at weekly intervals was tried on 20 patients with asthma and 28 patients with chronic dermatoses. The number of patients treated with each type of chronic dermatitis is too small to draw any definite conclusions. However, it is felt that further trial of this therapy in chronic contact dermatitis and chronic urticaria is indicated. The response to B_{12} by the patients with asthma was equivocal and in no case was spectacular.

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Editorial

THE SHIFTING PATTERNS OF ALLERGIC EXCITANTS

The list of causes of clinical allergic conditions is by no means fixed or static; it changes with the times. Etiologic factors fluctuate in importance as their incidence rises or falls. Nowhere is this more apparent than in the group of nonseasonal air-borne excitants which are so important in the causation of respiratory forms of allergy. As the nation has emerged from the agricultural into the manufacturing era, materials of animal and vegetable origin have been increasingly supplanted in the home as well as in the arts and industries by substitutes and replacement products of metals, synthetic resins, and synthetic fibers. Thus the chemist and manufacturer have eased the discomfort of many suffers of allergic coryza and bronchial asthma, especially, by creating acceptable, even superior, synthetic substitutes for the down, feathers, rabbit hair, goat hair, wool, cotton, kapok, and silk of beddings, furnishings, carpeting, and clothing. Silk threatens a mild resurgence, but obviously the trend is toward the diminishing use of animal and vegetable products. With many of its chief ingredients becoming less frequently used, the disturbing influence of house dust as an allergen is impaired.

Horse dander was at one time an allergic excitant pre-eminent in all strata of society, since contact with horses was rather universal. Now this allergen is important only to the very rich and to the rather poor, allergic individuals: to those who ride as an avocation and to those who tend the mounts or who drive milk wagons. In the great area of the motor driving and mechanized middle class, including city dweller and farmer, the horse rarely presents a respiratory problem. Nor does therapeutic serum from the horse provide the dilemma that it once did, since the diminishing use of this and other animal sera has lessened the incidence of serum accidents.

Not all materials of animal origin have lost ground as specific excitants. The danders of the dog and the cat are just as important as they were several decades ago, since the level of population of household pets has changed but little, being determined, not by the practicalities or expediencies of the Machine Age, but by human affections.

Mucilages and glues prepared from fish collagens, deadly agents for many allergic patients, are now less often encountered due to their replacement by adhesives of synthetic origin. Powdered orris root at one time enjoyed a pre-eminent position in the causation of respiratory allergic conditions since it was an important item in the formulas of many types of toilet powders. Now it is ignored by many cosmetic manufacturers, with a consequent drop in its importance as an excitant.

It would appear that even hay fever conditions have been influenced by the ebb of the agricultural era. Several forms, especially those due to ragweed

pollen, seem to have increased during the past half century due to enlarged acreages of hay fever-producing weeds resulting from the felling of the forests, the increase in marginal and abandoned farms, and the enormous network of railroads and highways with their uncultivated areas of rights of way, often choked with vegetation. The application of chemical sprays such as 2-4D just before the pollinating season may interrupt this trend in the future, as indeed it seems already to have done in New York City.

While the advances of the chemist and the manufacturer have lessened the plight of sufferers with respiratory allergies, they have also added tremendously to the discomfort of those whose chief allergic manifestations arise in the skin. A multiplicity of causative factors, unknown a few decades ago, are now quite common, having followed in the wake of the discovery of the newer chemicals and dyes used in the various industries and trades. The occupational forms of dermatoses have expanded the volume of allergic skin ailments to the point where these varieties outrank in occurrence many other clinical sensitizations and have tipped the scales of incidence in favor of these groups. Furthermore, in medical practice it is common to find that almost every newly introduced drug may cause allergic symptoms. Insulin, the sulfa group, and the antibiotics are outstanding examples.

The allergist of 30 years ago held in high regard excitants which are now of lesser consequence, and was only faintly familiar with many allergens which are considered of importance today; he could only speculate upon the fact that many disorders then of unknown origin were manifestations of drug, bacterial, and serum allergy. A watchful eye must be kept upon new products and new drugs, which may be actual or potential sources of allergic disturbance. Times change and we change with them, or should.

W. C. Spain, M.D.

PRESIDENT'S ADDRESS

THEODORE L. SQUIER, M.D., MILWAUKEE, WIS.

IN 1923 the Association for the Study of Asthma and Allied Conditions was organized. At essentially the same time the American Association for the Study of Allergy came into being. The founders of both societies had the same ideals. They had a common desire to promote better understanding of the causes of allergic diseases and to improve patient care. In 1944 these two societies combined to become the present American Academy of Allergy. Fundamental objectives of the parent societies have continued unchanged. These are, as stated in the constitution of the Academy, "Advancement of the knowledge and practice of allergy by discussion at meetings, by fostering the education of students and the public, by encouraging union and cooperation among those engaged in this field, and by promoting and stimulating research and study in allergy." During the salad years of the Academy these clearly stated purposes at times were obscured or even temporarily forgotten. Problems related to certification in allergy have been allowed to absorb energy and effort that could have been directed into more productive channels.

Some inequities and injustices in certification are known to exist. Uncompromising adherence to stated positions and heated recriminations have delayed the adjustments that surely will be made eventually.

It is the responsibility of the American Academy of Allergy to foster all efforts that promise to lead to improved training in allergy. Recognition of allergy through establishment of definite standards for training has resulted in better teaching in many schools and hospitals. It is equally true that in other institutions progress has been retarded by the continued disharmony and confusion that has existed among us. Since "He bids fair to grow wise who has discovered that he is not so," one may hope that the Academy as a scientific body will direct more of its energies toward scientific and educational objectives.

Techniques of diagnosis have been so emphasized that young men who wish to enter the field of allergy often fail to realize that a good allergist must be first of all a good physician. Knowledge of the significance of test reactions, discrimination in selection of methods of study to be employed in each clinical problem, and, above all, critical judgment in the evaluation of results are essential requirements imposed in the special practice of allergy. All of these particular requirements are in addition to the primary demand for sound understanding of general medicine and the knowledge required for whatever major medical specialty may have been elected. Special techniques are essential for the practice of good allergy. However, such special techniques can be used with wise clinical judgment only after broad basic knowledge of medicine has been first acquired.

The late A. H. W. Caulfield, who in 1933 was president of both parent societies, said he believed, "The practice of allergy at the present time is chiefly menaced in two respects. The first is the reporting of allergic manifestations in the individual or in a disease group when actually or probably they do not exist. The second is the overlooking of some other condition of more importance to the welfare and health of the patient than any symptoms referable to the detected atopens."

These truths are recognized by all good physicians who practice allergy. The careless, sensational, or even unprincipled practices of a very few men who never were good doctors but who call themselves allergists too often are looked upon as representative of allergy. They constitute unsavory exceptions which bring shame to all. Not long ago the dean of one medical school said, in relation to allergy, the importance of allergy as a specialty was recognized in his school. He added, however, that both he and his faculty were allergic to allergists. It is exceedingly unfortunate that in many medical schools and teaching hospitals the contribution to health and comfort which has been made through the study and sound practice of the principles of allergy is essentially unknown. Allergists are not exclusively to be blamed for the bad practices which are attributed to them. Too often internists and pediatricians with grossly inadequate training in allergy use the special methods of allergic management and attribute their inevitable failures to failures of allergy rather than to their own ineptness. The patients subjected to such inadequate treatment unfortunately are led to believe that further attempts at allergic care would be equally unsatisfactory. Internists and pediatricians who are loud in denunciation of allergy frequently are notable examples of the type of practice that we allergists deplore. This fact, of course, does not in any way excuse allergists who practice poor medicine.

For a number of years it has been my custom to preface lectures on allergy given to junior medical students with remarks made at one time by Francis Rackemann. They seem especially appropriate now. "The study of allergy," he said, "is only part of the broad field of internal medicine. Many phenomena observed in general medicine become understandable only when the underlying allergic mechanisms are recognized and understood. Further," he said, "allergic conditions should not be regarded as separate diseases, but rather as mere exaggerations of a normal response. The base on which allergy rests is becoming broader. It is evident that the study of allergy is not a narrow, specialized field, but one which requires broad understanding of the behaviour of the animal body in its efforts to rid itself of foreign proteins under many different conditions."

Reactions of hypersensitivity involve tissues and organs throughout the entire body. The study of allergic reactions has contributed and will continue to contribute to the understanding of the etiology and pathologic physiology of many diseases formerly regarded as of unknown origin. The immune reactions recognized in rheumatic fever, in periarteritis, in acute nephritis, and in similar conditions serve as examples of the ever widening scope of allergy. New techniques of study are constantly employed. Demonstration of the

major role which hypersensitivity plays in some forms of nephritis through use of tagged auto-kidney antibodies is one example. Allergy is no longer concerned solely with the limited group of conditions labeled as allergic diseases in older textbooks of medicine.

The tissues and organs which respond to allergic reactions are, however, subject to injury by many agents. As allergists we have acquired special knowledge of the responses attributable to antigen-antibody reactions. But, as clinicians, knowledge of the many normal and pathologic responses which occur independent of allergic reactions is likewise an inescapable requisite. Special training in allergy can never qualify a doctor to practice the specialty of otolaryngology. But an otolaryngologist can give his patients better care when he has added knowledge of allergy. Special training in allergy alone will never develop a qualified internist or pediatrician. But both the internist and the pediatrician who have acquired understanding of allergy will render better service to patients as a result of that added knowledge. Teaching of allergy should be included in the educational program of all young physicians entering those special fields of medicine.

Fellowship in the American Academy of Allergy is not, and should not be, limited to those who practice any one specialty of medicine. It is regarded as recognition of signal knowledge, interest, and experience in the field of allergy wherever applied. It is a most unhappy circumstance that there has been bitter controversy and schism over certification in allergy. The training and disciplines concerned with reactions of hypersensitivity are obviously applicable to all fields of medicine. There is no good reason why any specialty group, if it so desires, should not indicate by subcertification that certain qualified persons in that specialty have additional training in allergy. The corollary is obvious that allergy, if practiced honestly and well, is an added discipline applicable to many special fields.

The American Academy of Allergy has failed as an organization for the advancement of allergy in not insisting that every internist and pediatrician acquire some understanding of the fundamentals, at least, of allergy as one of the prerequisites for certification by those specialty boards. Responsibility for educational leadership in allergy rightly must be accepted by the Academy. Accordingly, immediate efforts should be directed toward establishment of at least minimum allergy training in every teaching hospital in the country. When this has been accomplished allergy will unhesitatingly be assigned its rightful place in medicine as an important adjunct to sound medical thinking and practice.

ROBERT A. COOKE—AN APPRECIATION

SOME twenty-eight years ago, on March 7, 1923, when allergy was still a term that was strange and not quite accepted, a little group of physicians met in New York with the intent of learning more about those mysterious and bizarre illnesses—hay fever, bronchial asthma, urticaria, and eczema. Those present were Drs. Arthur F. Coca, J. Alexander Clarke, Jr., Ruth Guy, Warfield T. Longcope, George MacKenzie, Edwin P. Maynard, Royce Paddock, Francis M. Rackemann, Oscar M. Schloss, Albert VanderVeer, Jr., Harry L. Alexander, and Robert A. Cooke, the first president of the group. The organization was christened the Society for the Study of Asthma and Allied Conditions and was an antecedent, a forebear, of the American Academy of Allergy. For many years, and in several capacities, Dr. Cooke has served well first the parent society, then the Academy. He was a member of the Executive Board of the original Society, and later a member of the Executive Committee of the Academy, and chairman of its Committee on Education. He is a member of the Editorial Board of the JOURNAL OF ALLERGY. No one has labored more diligently or more earnestly to create respect for the specialty of allergy or to enhance its reputation. He was a member of the original Standards Committee of the Society for the Study of Asthma, which later was replaced by the Subspecialty Board for Allergy of the Board of Internal Medicine and of Pediatrics; he was chairman of this group for many years.

He is pre-eminent as a pioneer in the field of allergy. Many of the accepted techniques of diagnosis and treatment, many of the methods of preparation of allergenic extracts, are the results of his investigations. His first article on allergy, a report of cases under observation and treatment since 1911, appeared in 1915. In 1919 he founded at the New York Hospital, then located on West 16th Street, the first clinic devoted entirely to allergic diseases. At the same institution, about 1923, he developed the first orientation course in allergy for practitioners and graduate students. He was a founder and first chairman of the New York Allergy Clinics Association, now the New York Allergy Society. He was the founder and is the director of the Institute of Allergy established in 1949 at the Roosevelt Hospital, where he has had charge of the Department of Allergy since 1932. He is the author of numerous scientific clinical articles and contributions upon various aspects of the allergic problem. He is National Consultant in Allergy to the Committee on War Time Graduate Medical Education.

Not all of Dr. Cooke's medical activities have been confined to the specialty of allergy. He has been a Governor of the American College of Physicians, later a Regent, and then a First Vice-President of the College of Physicians. He is an associate editor of *The Annals of Internal Medicine*. Outside the medical field he has been an Alumni Trustee and is now a Charter Trustee of Rutgers University, his alma mater, from which he received in 1925 the honorary degree of Doctor of Science.

Henry James, in his volume on Hawthorne, states that "The best things come, as a general thing, from the talents that are members of a group; every man works better when he has companions working in the same line, and yielding to the stimulus of suggestion, comparison, emulation. Great things of course have been done by solitary workers; but they have usually been done with double the pains they would have cost if they had been produced in more genial circumstances."

Such an idea has motivated Dr. Cooke's clinical and investigative career in medicine. He has always worked in association with others devoted to allergy and has proved a source of inspiration through his unfailing interest, enthusiasm, energy, and imagination. One of his greatest satisfactions should lie in the realization that this attitude and these ideas have been transmitted to many of the men he has trained, and have encouraged them to cling to the highest standards in practice and in investigation.

The Honorary Membership in this organization is awarded to those who, in the words of the Constitution of the Academy, "have made contributions of exceptional merit" in the field of allergy. Only five individuals have been so acclaimed. By offering this tribute we, the members of the Academy, wish to demonstrate our admiration of the achievements and our appreciation of the efforts, the time, and the experience which Robert A. Cooke has expended so generously upon our organization. There is no one whom we feel to be more worthy of this recognition. May he be with us for many years to come.

—W. C. Spain

Announcement

POSTGRADUATE COURSE

The American College of Physicians, in cooperation with the School of Medicine, University of Pittsburgh, will repeat a course on "Diseases Due to Allergy and Immune Mechanisms" on April 24 to 28, 1951, inclusive, in Pittsburgh, Pa. This course was originally given and oversubscribed in the spring of 1949 in Atlantic City. It was approved by the American Academy of Allergy and will be under the direction of Leo H. Criep, M.D. It is intended to serve the needs of trained allergists and internists. It will cover the most recent developments in Applied Immunology, hormonal relations, the chemistry of allergens, and clinical allergy. The faculty includes the following: Drs. Cooke, Mirsky, Kabat, Danonsky, Moses, Bohrod, Comroe, Rostenberg, Chase, Criep, Alexander, Tuft, Hoffman, Grauer and others. Each day will end with an informal round-table discussion, which proved so popular with the registrants the last time the course was given. All interested address Mr. E. R. Loveland, Executive Secretary, American College of Physicians, 4200 Pine Street, Philadelphia 4, Pa., at your earliest possible convenience.

Book Review

SINDROMES ALLERGICAS, by J. Cadrecha y Fernandez, M.D., Havana, Cuba, 1950, Cultural SA. 176 pp.

This little book written in Spanish shows an immense amount of careful study of the bacteria of the feces in over 400 Cubans suffering from various forms of allergy, including respiratory, in many instances with no history or findings of gastrointestinal abnormality. A similar study of over 50 normal controls is presented. Methods of culture of the stool flora, and preparation and standardization of the vaccines of such flora are fully given. The skin tests included foods and inhalants as well as the bacterial vaccine. Treatment consisted of these vaccines, sulfonamides, and dietary and environmental corrections.

The case reports show a high percentage of intradermal positive skin tests to cultures of the stool bacteria which were found, usually of the delayed type. Specificity of such findings is apparently indicated by a high percentage of good clinical results and the disappearance in many instances of the original positive skin tests and, occasionally, of the original offending organism or organisms in subsquent stool cultures. A study of a small number of apparently nonallergic occular conditions gives similar results.

The book is well written. All laboratory procedures are given in excellent detail. Further corroboration of these findings is of course essential, but the results suggest further trial of these methods.